NATIONAL INSTITUTE OF ARTHRITIS, DIABETES, AND DIGESTIVE AND KIDNEY DISEASES

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ANNUAL REPORTS

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National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases

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Z01 AM 13004-10 MRB
Z01 AM 13014-03 MRB
Z01 AM 13015-03 MRB
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TERMINATED PROJECTS (continued)

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Z01 AM 45029-04 CEB
Z01 AM 49001-11 PMB
Z01 AM 49004-05 PMB
Z01 AM 49005-05 PMB
Z01 AM 49007-03 PMB
Z01 AM 49008-02 PMB
Z01 AM 52002-07 GBB
Z01 AM 54018-08 DDB
Z01 AM 54019-08 DDB
Z01 AM 54021-07 DDB
Z01 AM 54028-05 DDB
Z01 AM 54032-03 DDB
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DIRECTOR'S SUMMARY

FY 1984 was the second full year during which the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (NIADDK) operated under its recently achieved Bureau status. The four major divisions of NIADDK, which previously had functioned as unofficial "clusters," have achieved new visibility in the biomedical research and voluntary health communities, as well as greater autonomy in making administrative decisions.

Since its establishment as a separate Institute at NIH, more than three decades ago, NIADDK has steadily expanded its research efforts in the prevention, diagnosis and better medical management of a wide range of disorders, mostly of a chronic nature. Several of these diseases are among the leading causes of disability and death in the nation; all affect seriously the quality of life of those suffering from them. They constitute a tremendous drain, both direct and indirect, on the human and economic resources of the United States.

The wide range of disorders coming within the purview of NIADDK include: arthritis and diseases of connective tissue; musculoskeletal diseases; skin diseases such as psoriasis and epidermolysis bullosa; diabetes, cystic fibrosis and other metabolic and endocrine disorders; digestive diseases such as peptic ulcers, cirrhosis and pancreatitis, nutrition and its relationship both to disease and well-being; diseases of the kidney and urinary tract, such as glomerulonephritis and urolithiasis and diseases of the blood.

Certain common scientific and biomedical denominators can be found in this broad array of diseases. These programs taken together encompass the various disciplines of internal medicine, less the cardiovascular system, allergy, and infectious diseases. Many of them overlap, with a common thread of endocrinology, metabolism, aberrant immune reactions, and nutrition running throughout. Basic research in endocrinology and metabolism has provided answers to questions in diabetes, and research directed at diabetes has led to new insights in other endocrine and metabolic diseases. The fields of metabolism and nutrition overlap. Nutrition and diet therapy play an indispensable role in the research, treatment, and prevention of diabetes (Type II), digestive diseases, and kidney and hematologic diseases. Moreover, in the case of certain metabolic diseases, diet therapy is the only effective treatment available. Autoimmune mechanisms are another major line of investigation connecting different programs at NIADDK. Aberrant autoimmune processes are suspected or implicated in such diverse diseases as rheumatoid arthritis, insulin-dependent diabetes, Crohn's disease, chronic active hepatitis, primary biliary cirrhosis and the glomerulonephritides.

The outlook for advances in the treatment and prevention of these diseases is dependent on basic and clinical investigations into the nature of their interference with the normal functioning of the body's many complex systems. In order to carry out this mission, NIADDK supports research and training projects in more than 400 non-Federal institutions. Intramural basic and clinical research is also conducted on and near the NIH reservation in Bethesda, Maryland, and at field stations in and near Phoenix, Arizona.

Equal Employment Opportunity Program

A major focus of the EEO Program in 1984 has been the implementation of the multi-year Affirmative Action/Federal Equal Opportunity Recruitment Program (AAP/FEORP) Plan. The Plan calls for special recruitment measures to make the NIADDK work force representative of the nation's population. These measures are to be taken to correct the underrepresentation of minorities and women in the following occupations:

Series	Occupation
GS 303	Miscellaneous Clerk/Assistant
GS 318	Secretary
GS 322*	Clerk Typist
GS 334*	Computer Specialist
GS 341	Administrative Assistant/Officer
GS 401	Biologist
GS 404	Biological Laboratory Technician
GS 601	Health Scientist Administrator
GS 602*	Medical Officer
GS 1035	Writer-Editor/Public Affairs
GS 1101	Grants Management Specialist
GS 1102	Contracts/Procurement Specialist
GS 1320	Chemist

(*Applies to handicapped individuals and disabled veterans only)

Responsible officials have been designated for carrying out the plan, and copies of the AAP/FEORP document have been disseminated to all Bureau supervisors. A report was completed in Fall 1983 which assessed the Bureau's progress toward this end.

A Sexual Harrassment Policy Statement and Plan for prevention is also included in accordance with NIH and DHHS directives.

Internal Initiatives:

The Bureau supports the development of disadvantaged minority students through the Stay-in-School program which allows them to work part-time while pursuing their education. Short-term experience is also provided through the Summer Program. Such positions are located in both administrative and scientific areas.

The Bureau continues to publish a monthly newsletter through the EEO Office apprising all Bureau employees of matters of importance or special interest in this area. Personnel policies and procedures are explained in lay terms with the advice and consultation of the Institute's Personnel Branch.

The Bureau's Federal Women's Program Manager sponsors developmental programs for its employees through forums such as the Annual Secretaries' celebration, featuring speakers on topics of interest. In response to a survey of support staff, a series of lunchtime seminars were conducted. Topics included financial planning, scholarship assistance, and selfdevelopment.

Tissue culture classes continue to be offered in the Division of Intramural Research for all interested employees who wish to pursue a career or expand their skills in the field of biomedical science. In addition, a new course in biotechnology approaches and techniques met with overwhelming interest in its first semester (Spring 1984).

The Bureau has reestablished its EEO Advisory Committee. This committee will serve as a direct link with the employees to advise the Director of their concerns and to make recommendations to improve their status and career opportunities.

The Bureau completed a Public Health Service Qeustionnnaire on barrier analysis to identify impediments to the employment of American Indians and Hispanics. The bureau is represented on the NIH Hispanic Advisory Committee and recruitment efforts are being focussed on this severely underrepresented group.

A further effort to ensure fairness in hiring procedures is made by including the EEO Officer on Qualifications Review Boards and Search Committees, particularly when filling vacancies for senior level positions in the Bureau.

Outreach Activities:

The Bureau supports the Minority Biomedical Research Support (MBRS) Program through an intra-agency agreement with the Division of Research Resources. The support began at a very modest level of $400,000 the first year, increasing to $1.25 million by 1984. The Program supports 41 projects through 13 separate research and training awards.

The Minority Access to Research Careers (MARC) Program interagency agreement with the National Institute of General Medical Sciences allows the Institute to increase the number of minority scientists engaged in biomedical research. Funds are available for pre-doctoral faculty fellowships, visiting scientists, and honors undergraduate training.

A function of the EEO Office includes active participation in the NIH Contract Compliance Program, a component of the NIH Civil Rights Program. Its purpose is to 1) educate staff, 2) collect data, and 3) provide technical assistance relating to compliance under executive orders, titles, regulations, and civil rights laws, as they pertain to the contracting and grants staff.

The Bureau is committed to ensuring an equal opportunity to ethnic minorities and women in contributing to biomedical research through the Extramural

Associates Program (EAP) which offers temporary appointments at NIH to personn from minority and women's academic institutions. The EEO Officer participates in the EAP through representation on its Quarterly Review Board and acts as an advisor to the program.

In addition to participation in local recruitment and training programs, NIADDK scientists and professional staff participate in the NIH Visiting Scientist Program, where Bureau staff may teach or advise students and faculty at the host institution for a specified period of time on careers in the biomedical sciences.

Outreach activities include visits to the NIADDK laboratories by students from local high schools and colleges. Staff scientists prepare presentations and conduct tours of the facilities and, in some cases, provide demonstrations of sophisticated equipment used in current biomedical research.

This year the Bureau helped sponsor the 41st Joint Annual Meeting of the National Institute of Science, the Beta Kappa Chi Scientific Honor Society and the Brookhaven Semester Program. Support was provided in the form of NIADDK funds for student travel and awards.

A Journal Distribution Program is operated out of the EEO Office through which used scientific books and journals, donated by NIH staff, are collected and distributed to various minority and women's colleges and universities. These contributions enable colleges to better prepare minorities and women for successful careers in the sciences.

ANNUAL REPORT FOR THE

OFFICE OF PROGRAM ACTIVITIES AND EVALUATION

The Office of Program Activities and Evaluation (OPAE) is reponsible for activities pursued by the Bureau in the areas of technology assessment and technology transfer, inventions and patents, disease prevention, program evaluation, orphan drugs and research animal welfare. OPAE also provides staff support to the immediate Office of the Director, NIADDK, particularly with respect to Bureau-wide dissemination of policy and information presented to senior staff. OPAE is the point of liaison with several NIH offices and committees, including the Office of Medical Applciations of Research (OMAR), the NIH Office of Disease Prevention, the NIH Office of Program Planning and Evaluation (for evaluation-related activities), the NIH Prevention Coordinating Committee, the NIH Orphan Drugs Committee and the Trans-NIH Coordinating Committee for Research Animal Resources. The Office of Program Activities and Evaluation is also responsible for the preparation of the Annual Report of the Director, NIADDK. This latter document provides an overview of Bureau operations and activities, specific program efforts, and research accomplishments and directions.

OPAE is also responsible for the coordination and submission of Congressionally mandated periodic reports which are required of NIADDK. Among these are annual reports of the interagency coordinating committees for arthritis, diabetes, and digestive diseases, and annual evaluation reports for the Institute's Multipurpose Arthritis Centers and Diabetes Research and Training Centers. In 1984 the office prepared the Institute Director's Opening Statement for the FY 1985 appropriations hearings. Also this year, OPAE has prepared the opening sections of this Annual Report as well as those of the four extramural divisions of the Institute.

Disease Prevention

The Office of Program Activities and Evaluation is the focal point of Bureau efforts related to prevention. These include coordination of NIADDK participation in special prevention initiatives, data analysis, periodic and special reporting, information dissemination, sponsorship of conferences and external representation.

OPAE performs surveys of ongoing Bureau operations related to disease prevention and also performs an ongoing analysis of NIADDK's active grants and contracts portfolio with a view to identifying those with a significant prevention component. The most recent review of active grants and contracts was completed in 1983. Also in 1983, OPAE made an analysis of a large representative sample of grant applications (those submitted for review during the June 1983 National Advisory Council meeting) to establish how prevention-related grant applications fare in study section review as compared to nonprevention-related grant applications. The analysis demonstrated that, within the fundable range of priority scores, the former did just as well as the latter. In addition, OPAE is developing a prevention coding system so that future research grant awards with such a component may be identified at the time of award. This activity is being carried out in close collaboration with the NIADDK Office of Program Planning and Analysis and Division of Extramural Activities.

Working closely with the various Divisions of NIADDK, OPAE identifies research topics with possible application to disease prevention. These include:

- Osteoporosis
- Prevention of the Onset of Type II Diabetes
- Prevention of the Complications of Type I Diabetes
- Prevention of Recurring Peptic Ulcers
- Prevention of Dwarfism and Normal-Variant Short Stature
- "Sports Medicine" (Prevention of injuries)
- Prevention of Diverticulosis and Diverticulitis
- Prevention of Recurring Cholelithiasis
- Prevention of Obesity
- Prevention of Renal Disease Associated with Analgesic Abuse
- Diseases That Can Be Prevented by Nutritional Intervention
- Prevention of Recurring Urolitiasis

OPAE provides prevention-related materials for inclusion, where called for, i Bureau and NIH documents. Examples of this include the preparation of a secti on prevention and related activities for the annual NIADDK budget narrative; submission of Bureau-specific material to the NIH Office of Disease Prevention for inclusion in the NIH Research Plan; a section on prevention in the opening statement of the NIADDK Director at Congressional appropriations hearings; a special chapter on prevention-related research in the Annual Report of the Director of NIADDK; and preparation of NIADDK's portion of the Annual NIH Report on Disease Prevention, and in the biennial Department Report on Disease Prevention.

Program Activities

OPAE is responsible for preparation of the Congressionally-mandated Annual Report of the Director, NIADDK. The Second Annual Report (covering FY 1982) was transmitted to the President and to the Congress within 60 days of the close of FY 1982. Like its predecessor, this volume provided an overview of the Bureau research and training efforts in the previous year, emphasizing initiatives and accomplishments in all its Divisions. A final printed and illustrated version was received from the Goverment Printing Office in the winter of 1983 and has been disseminated broadly to the Bureau's diverse professional and lay constituencies as well as to specific Congressional Committees, members of the House and Senate, and appropriate officials in the Executive Branch. Like the first report, it resulted in considerable laudatory comment. The Third Annual Report (covering FY 1983) was transmitted to the President and to the Congress within 60 days of the close of FY 1983. This Report will be printed by the Government Printing Office, and it is hoped that the printed and illustrated version can be disseminated on a large scale in the Fall of 1984. Preparation of the Fourth Annual Report of the Director is underway at present.

The Office of Program Activities and Evaluation provides direct staff support to the Director, NIADDK, for a wide range of activities. These include preparation of the record of the bi-weekly meeting of the Associate Directors of the Bureau and dissemination of the resulting policies and information to relevant Institute staff. OPAE also performs liaison duties for the Institute with the Office of Medical Applications of Research (OMAR) for consensus conferences sponsored by NIADDK, and for technology assessment related to inventions, patents, and, ultimately, Medicare reimbursement for specific medical technologies. OPAE also provides liaison with respect to the NIH Orphan Drugs Committee and the Trans-NIH Coordinating Committee for Research Animal Resources.

Evaluation

Evaluation activities at NIADDK are intended to assist program managers in finding solutions to problems that are important to them, and thereby help achieve improved program management. From this standpoint program evaluations can consist of undertakings other than the traditional projects. Such projects, however, are certainly important and, in the past, have provided the support necessary to produce valuable reports detailing accomplishments and needs in specific NIADDK categorical disease research programs. One such report was the "Analysis of Research Needs and Priorities in Dermatology," which was produced with evaluation set-aside funds (the traditional source of funding) and published as a supplement to the November 1979 issue of the Journal of Investigative Dermatology. The project, the so-called "Odland Report," had been initiated under a mandate of Congress. In 1982, an update of the report was formally requested by Congress. An alternative source of funding (other than set-aside funds) was identified and the report, entitled "Research Needs and Priorities in Dermatology - Update 1984," will soon be printed and distributed in its final form. This is another illustration of the flexible context in which evaluation studies can be performed - it is the use to which they are put that defines them, rather than their means of funding.

In addition to the *ad hoc* evaluation activities such as those described above, the Office of Program Activities and Evaluation serves as liaison with the NIH Office of Program Planning and Evaluation with respect to evaluation set-aside activities mandated by the Public Health Service Act. This has included, among other things, preparation of NIADDK's Annual Evaluation Plan for inclusion in the overall Evaluation Plan prepared by NIH each fiscal year.

The following evaluation studies, funded through the set-aside mechanism, were distributed in their final form during the past year:

1) "Research Needs in Hematology"

2) "Evaluation of the Musculoskeletal Diseases Program"

Technology Assessment and Transfer

The Office of Program Activities and Evaluation is the locus of technology assessment and technology transfer activities at NIADDK. Included among these is the provision of state-of-the-art information to OMAR for transmittal to the

Office of Health Technology Assessment in the National Center for Health Servi Research, which develops the Public Health Service assessments of, and provides advice to, the Health Care Financing Administration regarding Medicare coverage for medical, surgical, and diagnostic technologies. This information includes computerized bibliographies of the relevant scientific literature and review of relevant information. Requests to OPAE during the past year have included the following topics:

- Percutaneous and Noninvasive Lithotripsy by Ultrasound and Other Modalities in the Treatment of Kidney Stones.
- Dual Photon Absorptiometry
- Photodensitometry (Radiographic Absorptiometry)
- Injection Sclerotherapy for the Management of Bleeding Esophageal Varices
- Apheresis in the Treatment of Scleroderma
- Apheresis in the Treatment of Polymyositis
- Apheresis for Acute Stage Rejections of Renal Transplant
- Apheresis Used in Preparation for Kidney Transplant
- Electrocoagulation for the Treatment of Gastrointestinal Hemorrhage
- Portable/Wearable Artificial Kidney System

OPAE has also reviewed and commented on drafts of the following Public Health Service Assessments:

- $^{13}CO_2$ Breath Test for Diagnosing Fat Malabsorption
- $^{13}CO_2$ Breath Test for Diagnosing Bile Acid Malabsorption
- Lactose Breath Hydrogen Test for the Diagnosis of Lactose Malabsorption
- Lactulose Breath Hydrogen Test for Small Bowel Bacterial Overgrowth and Small Bowel Transit Time
- Transplantation of the Pancreas
- Apheresis in the Treatment of Goodpasture's Syndrome
- Apheresis Used in Preparation for Kidney Transplant

The Office of Program Activities and Evaluation also serves as the Bureau liaison with OMAR regarding patent rights to inventions arising out of research supported by NIADDK. Some recent inventions for which OPAE obtained expert advice regarding patentability were:

- Dual Photon Absorptiometry
- A Simple Method for Uniquely Releasing Periplasmic Proteins from Bacterial Cells
- Luciferase/Luciferin-coupled Membrane ATPase Assay
- A Human Male-Specific Repeated DNA Sequence Suitable for Sex Determination
- Lyophilized Hematin for Intravenous Administration
- Macromolecular Crowding Allows Blunt-End Ligation by DNA Ligases from Rat Liver of _E. coli_
- A Serum or Plasma Test for the Detection of Cancers
- Calcitonin for Oral Administration
- Prevention and Treatment of Osteoporosis with Promethazine HCl
- Calcitonin Incorporated into Liposomes
- Isolation of Skeletal Growth Factors of Varying Molecular Weights from Bone of Several Species
- Enzymatic Generation of Native Cross-link Intermediates in Helical Domains of Collagen
- Protection of Non-Steroid Anti-Inflammatory Drugs Induced Gastric Mucosal Damage by the Polyamines
- Method for Inhibiting the Secretion of Stomach Acid with Secondary Amines
- Carbamates of Deacetylcolchicine, Thiodeacetylcolchicine and their N-Methyl Congeners
- Potentially Implantable Glucose Sensor

Consensus Development Conferences

Office responsibilities with respect to technology assessment and transfer also include the NIH Consensus Development Conferences, pertaining to both prevention and other areas within the purview of NIADDK. Such conferences are initiated and coordinated by OPAE, in conjunction with OMAR. On February 27-29, 1984, NIADDK and OMAR held a Consensus Development Conference devoted to Analgesic-Associated Kidney Disease. On April 2-4, 1984, NIADDK, in conjunction with OMAR, held a Consensus Development Conference on the Prevention and Treatment of Osteoporosis. The latter was a particularly timely conference and the resulting consensus statement attracted considerable attention in the medical literature and lay media. Less than a month after the consensus panel had urged that estrogen replacements "should be considered" for post-menopausal women at risk, the FDA announced approval of estrogen for treatment of osteoporosis. At present OPAE is organizing a Consensus Development Conference, in cosponsorship with the National Heart, Lung and Blood Institute, and OMAR, to be held February 11-13, 1985, on Health Implications of Obesity. A promising scientific program has been assembled for this conference.

ANNUAL REPORT FOR THE
OFFICE OF PROGRAM PLANNING AND ANALYSIS

The Office of Program Planning and Analysis (OPPA) is responsible for advising the NIADDK Director and senior staff on selected policy issues and for providing leadership and guidance for Institute activities in planning, program and policy analysis, and legislation. This office also serves as central Institute liaison to offices and committees concerned with planning, analysis, and legislation at NIH and other Federal agencies.

Planning

During FY 1984, OPPA developed and issued guidelines for program planning, and assisted the Divisions in implementing these guidelines. The guidelines focused on program initiatives for meeting research needs and pursuing research opportunities. Other sections of the plan describe program goals and accomplishments and other background information.

OPPA is responsible for developing and refining the Institute's program planning process. Each fall, OPPA arranges meetings for Division staff to present to the NIADDK Director preliminary drafts of the research advances and initiatives of their implementation plans. Also, each fiscal year, OPPA is responsible for assembling an Institute-wide implementation plan and for arranging the plan's review by NIADDK Council.

NIADDK recognizes that a successful planning process depends upon participation of individuals with the necessary expertise. Each Division collaborates with a broad spectrum of individuals and groups from outside the Institute in identifying research advances, opportunities, and initiatives, as well as in assessing broader policy issues regarding research resources and program balance. OPPA assists this process when possible, particularly by preparing trans-Institute analyses of aspects of the plan for presentation to reviewers.

OPPA makes extensive use of the extramural Divisions' descriptions of research advances and program initiatives in reporting to a variety of audiences, including NIH and Departmental staff, other Administration officials, members of Congress and their staffs, biomedical researchers, and public interest groups. Information from these documents is incorporated into budget justifications, testimony, appropriations briefing materials, the NIH research plan, and various other analytic reports. The plans also provide a solid basis for program accountability and evaluation.

Among OPPA planning goals for the coming year are to:

- Improve the ease of adapting material from the plan for use in other analytic documents.

- Promote accessibility to the wealth of research advances documented by Division staff.

- Expand OPPA's store of data on NIADDK-related diseases and disorders.

- Provide prompt transmittal of OPPA reports and analyses through newly installed, highly sophisticated word processing and personal computer equipment.

Program Analysis

OPPA staff continually receives and responds to many program analysis requests. A response to these requests typically requires a review of the entire Institute´s research programs in order to determine Institute involvement in the subject area or disease in question. Responses generally include explanatory narrative material, itemized listing of funds expended and/or projected, and a listing of grants and intramaural projects relevant to the subject of the request.

OPPA is responsible for generating all NIADDK external reports which require an analysis of scientific programs. The report generation method involves a computer search of CRISP files for projects coded under a given scientific category. The list of projects is then reviewed by appropriate program directors, and any changes are incorporated into the final report. A major goal is to reduce the reporting burden placed on NIADDK program directors and to develop accurate, reproducible, and consistent reporting methods for the Institute.

Legislative Analysis

OPPA has primary responsibility within NIADDK for advising the Director and senior staff concerning Congressional interests and actions relevant to the Institute´s organization, policies, and programs. During the second session of the 98th Congress, OPPA followed a number of legislative issues including animal welfare, disease prevention, organ transplantation, and nutrition. Of particular interest to the Institute is legislation that would remove the arthritis, musculoskeletal and skin diseases programs from NIADDK and reconstitute them as a separate NIH institute. This measure--first introduced in the 97th Congress--was incorporated in Senate and House bills (S. 773 and H.R. 2350) to extend NIH expiring authorities--including NIADDK centers, national advisory boards, and other special activities--as well as in two smaller bills (S. 540 and H.R. 1403). All four bills also contain provisions that would require a study of the entire NIH organizational structure by the Institute of Medicine, as well as an administrative review of NIADDK´s organization by the Secretary, subsequent to creation of the separate arthritis institute.

In addition to legislative analyses of these and other issues, OPPA played a key role in the appropriations process, including the development of briefing documents related to Congressional directives and issues relevant to NIADDK. OPPA participated in preparations for several briefings of Congressional staff who arranged visits to the NIH, and also provided Institute-wide guidance for responding to Congressional inquiries.

OFFICE OF HEALTH RESEARCH REPORTS

The Office of Health Research Reports (OHRR) writes, produces and disseminates information about the varied research and programs of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (NIADDK). This information takes many forms: announcements, news releases, research highlights stories and notes, reports to Congress, brochures, pamphlets, fact sheets, background papers, responses to public and congressional inquiries, exhibits at scientific meetings, and importantly, contact with representatives of the print and broadcast media and of voluntary and professional associations.

CONGRESSIONAL INQUIRIES

The Office of Health Research Reports coordinates and produces responses to all "controlled" correspondence referred to the Institute from Congress, the Department and the White House. Because of active public interest in a wide-ranging number of subjects within its research purview, NIADDK receives a large volume of these requests.

From June 1, 1983, to May 31, 1984, OHRR prepared 225 written responses to inquiries from Senators, Congressmen, the Secretary of HHS, and the White House, a 22 percent increase over last year.

OHRR also responded to over 100 telephone inquiries from congressional staff. These are noted on "records of call" and distributed to appropriate Institute staff members.

PUBLIC INQUIRIES

A large portion of the U.S. public is directly concerned with the disease areas under investigation by NIADDK. At least 36 million Americans have some form of arthritis, 11.5 million have diabetes, 20 million have chronic digestive disorders, and 25 million have skin diseases significant enough to visit a physician. Kidney diseases still claim the lives of 45,000 people in the U.S. each year.

From June 1, 1983, to May 31, 1984, OHRR received and responded to 4,388 telephone inquiries from patients, their relatives and friends, students, allied health professionals, physicians, and scientists. In addition, OHRR staff wrote 513 letters in response to public inquiries, exclusive of requests for publications or congressional inquiries, in spite of increasingly effective means of responding, such as the development of fact sheets and backgrounders and the identification of suitable materials from voluntary organizations and the media.

Requests for information dealt with questions about accepted diagnosis and treatment procedures, controversial forms of therapy and proposed "cures," locations of appropriate researchers, sources of financial assistance, and how to become a patient in NIADDK research studies.

PRESS ACTIVITIES

Contact with representatives of the print media is an integral part of OHRR's public information program. In support of the established policy to provide information about NIADDK's programs and activities, OHRR reaches a variety of media outlets through personal contact and distribution of research highlights stories, research notes, feature stories, announcements, and news releases.

OHRR prepared several major announcements and feature stories in 1983-84, including: Dr. Pierre Renault Appointed Deputy Director; Potential New Treatment for Arthritis Studied; Older Women May Need Estrogen Replacement and Additional Calcium to Prevent Osteoporosis; Pancreas Cells Studied on NASA Space Shuttle; New Pamphlet on Prevention and Treatment of Kidney Stones; Virus May Be the Cause of Rheumatoid Arthritis; 250 People Enrolled in Nationwide Study on Control of Diabetes; Long-Term Prognosis for Biliary Atresia Patients; New Report of Three-Year Needs in Blood Research; Long-Term Ranitidine Treatment Can Control Massive Gastric Acid Secretion; Researchers Relate Liver Failure to Brain Dysfunction; Protective Layer of Yeast Identified - May Aid in Fighting Infections.

An important aspect of media relations for OHRR concerns major scientific meetings held throughout the year. OHRR staff cover appropriate meetings and make contact with reporters, public relations staffs of private health organizations and NIADDK grantees. When possible, the office tries to identify and write up research presentations in advance of these meetings. This year, for example, OHRR prepared five research highlight stories for use in the press room at the annual digestive disease scientific sessions.

OHRR is also continuing to emphasize the achievements of the Division of Intramural Research (DIR), both clinical and fundamental research units. OHRR staff prepared eight stories on prestigious awards to DIR scientists, as well as several of the research stories listed above. OHRR staff have widened their outreach to DIR to include the laboratories located in Phoenix, Arizona, and have done extensive interviews in preparation for several upcoming news and feature articles.

"Research Notes" is a new project of OHRR. It is a set of brief research write ups, at least one per Division, produced at irregular intervals several times a year. The "Notes" are mailed to the OHRR press list as story ideas and to NIADDK constituencies, including the Council, advisory boards and relevant voluntaries and professional groups to keep them informed of highlights of NIADDK-supported research. The "Notes" reflect the range and diversity of NIADDK research in two or three pages.

OHRR continues to distribute to senior staff regular issues of "NIADDK Current Clips from the Printed Media," including special editions when there is intense coverage of a topic (such as an NIADDK consensus conference). In addition to reviewing and clipping newspapers and magazines that are received by the office, OHRR has contracted with a clipping service so that we can monitor coverage of NIADDK concerns in a broader fashion.

RADIO AND TELEVISION

Several Institute scientists were featured this year on radio and television broadcasts, both locally and nationally. These broadcasts included radio interviews on alopecia, adult acne, and osteoporosis, which were broadcast on WGMS locally and provided to stations across the country.

In addition, OHRR staff arranged for a videotape of intramural diabetes research for use by the Juvenile Diabetes Foundation and produced an audiotape for a radio show on hypoglycemia. OHRR staff also work closely with television personnel who come to interview NIADDK extramural or intramural staff, for example, on lupus research or on Japanese scientists at NIH.

THE NIH RECORD

The NIH Record is a primary source of communication with NIH employees, although it is also distributed to wire services, local news media, science writers and editors, and scientists in the United States and abroad. OHRR produced 42 NIH Record stories this year, including 5 cover stories. Topics included research and feature articles, appointments, retirements, awards, and notices.

SPECIAL PROJECTS

This year NIADDK sponsored two NIH consensus conferences with the Office of Medical Applications of Research. OHRR staff were actively involved in both, developing materials to advertise the program to appropriate audiences and pursuing media contacts before, during and after the conference. In February 1984 the conference concerned analgesic-associated kidney disease. OHRR staff made special efforts in the weeks before the meeting to obtain preconference publicity through radio and television interviews of speakers. Media interest was sigificant, especially because the panel statement included the recommendation that "serious consideration" be given to removing combination painkillers, popular drugs, from the market. Twenty-four reporters attended the conference, which received widespread print coverage and was discussed on local and national television.

Interest in the second consensus conference, held in April 1984 on osteoporosis, was even more intense. The audience was the largest ever to attend a consensus conference; fifty-one members of the media registered and stories appeared on local and national television. Approximately 60 news clippings have been gathered. Interest from both the lay and trade press continues to be steady.

OHRR staff have worked closely with NIH staff who prepare Healthline, a set of articles for lay people on progress in medical research. This year Healthline published two issues devoted exclusively to NIADDK, across the Divisions, and a third issue of several stories on digestive diseases, timed to coincide with Digestive Diseases Week in May.

OHRR continues to cooperate with voluntary and professional associations such as OHRR distribution of materials, preparation, and joint dissemination of pamphlets in specific disease areas, e.g., Paget's disease and epidermolysis bullosa, and preparation of articles and notices for newsletters. One OHRR staff member was recognized by the American Lupus Society for her special efforts to provide lupus information. The office is making stronger efforts to reach these organizations by mailing releases and publications to headquarters staff and their chapters and to specialized trade publications.

Several special projects were undertaken in 1983-84 on behalf of the Division of Intramural Research. These included the writing and production of a cystic fibrosis recruitment brochure and of a patient education fact sheet on hyperparathyroidism. In addition, OHRR provided publicity for a new series of Tuesday morning lectures started last fall and presented by intramural scientists.

Efforts with clearinghouses included OHRR participation in the PHS Coordinating Committee for Clearinghouses and coordination of two major panel reviews of NIADDK clearinghouses by PHS/HHS. OHRR regularly updates Clearinghouse advisory boards on policies of PHS and HHS that affect their operation. OHRR is providing advice and support to the National Digestive Diseases Education Program, a group of private and Federal organizations.

EXHIBITS

For many years, OHRR has had responsibility for coordinating the exhibits program for NIADDK, designed primarily for a scientific audience. In 1983-84, OHRR had a scientific exhibit at the annual Lupus Foundation meeting and developed an exhibit for the American Diabetes Association meeting with an accompanying administrative guide describing the programs of the Division of Diabetes, Endocrinology and Metabolic Diseases. NIADDK clearinghouses continue to actively exhibit at numerous scientific and professional education meetings throughout the country, and OHRR publications are distributed with clearinghouse materials.

PUBLICATIONS PROGRAM

OHRR continues to have Institute-wide responsibility for the approval, production and printing of publications. As in previous years, detailed Publication Plan Clearances are submitted to NIH and the Department. This year the Concept Review Plan was added as a requirement for publications as well. From June 1983 to June 1984, eighteen concept clearances were processed and approved. Two concept clearances that had been submitted prior to June 1983 were approved, one concept clearance was denied (on the grounds that the information would be dated and very limited in audience appeal), and fourteen have been submitted but not yet returned.

In the area of materials for the public, OHRR continues to identify and obtain existing informational materials from a variety of non-Government sources, particularly private health organizations. Where information "gaps" exist in subjects of public interest, OHRR will prepare materials as needed. For example, a new pamphlet entitled Prevention and Treatment of Kidney Stones was printed this year and promoted through health and science writers. The Diabetes Dictionary was a joint project of the diabetes clearinghouse, in cooperation with the American Diabetes Association, the Juvenile Diabetes Foundation, and the American Association of Diabetes Educators, as well as the Eli Lilly Company, which underwote printing costs. At least 15,000 copies of this publication have been distributed. Osteoporosis: Cause, Treatment, Prevention was printed in 1983 and was promoted through a brief announcement to science and health writers and Arthritis Foundation chapters and other organizations for musculoskeletal disorders. Nevertheless, the supply of 30,000 was exhausted in eight months and a revision is being prepared, based on the results of the consensus conference. Several Dairy Council regional groups have reprinted the pamphlet.

Publication and editorial assistance were provided to program personnel for: A Report to the House Committee on Appropriations: Lupus Erythematosus Research; Initiatives for Arthritis: Progress and Future Directions--A Report of the National Arthritis Advisory Board; Evaluation of the Musculoskeletal Diseases Program; Educational Materials for and about Young People with Diabetes; Diet and Nutrition for People with Diabetes; Second Annual Report of the Director, NIADDK; and Research Needs in Hematology. The second annual director's report and the kidney stones brochure received Blue Pencil Awards from the National Association of Government Communicators.

ANNUAL REPORT

DIVISION OF ARTHRITIS, MUSCULOSKELETAL AND SKIN DISEASES

ARTHRITIS RESEARCH AND TRAINING PROGRAMS

Overview

The content of the research effort in arthritis can be considered in four general areas that consume roughly equal portions of the program budget. The first of these includes the projects associated with immune disturbances, autoimmune diseases, rheumatoid factor, antigen-antibody reactions and immune complex formation. The second area consists of research concerned with collagen, connective tissue, basement membranes, synovial fluid and articular cartilage. The third area consists of research projects in rheumatic diseases; rheumatoid arthritis, degenerative joint diseases, systemic lupus erythematosus, gout, heritable disorders, and many others. The fourth area concerns research associated with the structure, function, contraction, and metabolism of skeletal muscle.

The program continues to be separated organizationally as Arthritis 1A (Arthritis Program) and Arthritis 1B (Muscle Biology Program) to give visibility to rheumatic disease activity and skeletal muscle research as distinct entities. Annual data summaries now display the total number of projects and dollars obligated for each of these major components of the program.

Evidence for a Viral Origin of Rheumatoid Arthritis in Man

Parvoviruses are insidious pathogens whose relation to human disease has remai obscure since they were first recognized in different animal species. They are often "silent" in cell culture systems or laboratory animals, and can persist at the molecular level as integrated inserts in chromosomal DNA. It recently has been reported that parvovirus-like agents can be isolated from the synovial tissue of patients with severe rheumatoid arthritis (RA).

In a study leading to the identification of these agents as viruses with universal properties and a possible link to RA, long-term cultivation of rheumatoid synovial cells with WI-38 human lung fibroblasts was accompanied by the transient appearance of microfoci of piled-up, aggregated cells. Electron microscopic examination of these cells revealed rare intracytoplasmic budding particles with long surface projections. Subsequent inoculation of culture extracts into the brains of newborn mice led to the emergence of an agent that was lethal in mice, and on the basis of inconclusive evidence was tentatively regarded as an enveloped RNA virus. This agent, called RA-1, was identified as a DNA parvovirus based on completed virological studies and ongoing biochemical studies.

Extracts from the mouse brains infected with the RA-1 virus were then injected into rabbits, thereby producing antisera with high virus-neutralizing activity. These antisera have allowed the use of sensitive enzyme-linked immunosorbent assays (ELISA), which enable the detection of antigenically related viruses in the synovia of different RA patients, as well as help provide for understanding the possible relation of RA-1 virus to other viruses. To date, 13 of 14 synovial specimens from RA patients have shown antigenic reactivity to RA-1 in ELISA tests.

Although these findings do not conclusively define the link between RA-1 and other parvoviruses, and rheumatoid arthritis, they do seem to suggest that they may have a role. Further research likely will elucidate this.

Disease-modifying Agents Such as Oral Gold and Methotrexate Appear Promising in the Treatment of Rheumatoid Arthritis

Over the years numerous substances or therapies have been attempted as disease-modifying agents in the treatment of rheumatoid arthritis (RA). Gold salts have been used to treat the symptoms of RA for 50 years, but until recently this therapeutic modality required deep intramuscular injections on a weekly basis. More recently, an orally administered gold preparation, auranofin, has been used. In a recent prospective, controlled, double-blind multicenter trial, a comparison was made of placebo, auranofin, and parenteral gold sodium thiomalate in 209 patients with active RA.

The trial was performed over a period of 21 weeks, with 161 patients completing at least 20 weeks of treatment.

Results indicated that there was statistically significant improvement in the median number of tender joints, the "joint pain/tenderness score", and physician assessment of disease activity for both gold treatment groups, although this improvement was modest. However, gold treatment would seem to be an effective treatment modality for patients with active, responsive RA disease.

In a prospective, randomized controlled trial of low dose pulse methotrexate (MTX), 189 patients to date with defined RA who did not respond to parenteral gold and/or D-penicillamine have been treated. Fifty-seven of 95 patients assigned to MTX and 53 of 94 on placebo completed 18 weeks of treatment. On a number of parameters (pain/tender joint count, pain/tender joint score, swollen joint count, swollen joint score, duration of morning stiffness, patient assessment of improvement, physician assessment of improvement, erythrocyte sedimentation rate) patients showed frequently dramatically greater response on MTX then on placebo. However, adverse drug reactions led to the withdrawal of 29 patients on MTX and 11 on placebo; elevated liver enzymes accounted for the withdrawal of 18 MTX patients and 4 placebo patients; 2 patients on MTX developed pancytopenia and 2 developed leukopenia.

It thus appears that methotrexate, administered in low weekly doses, is an effective but potentially toxic treatment for rheumatoid arthritis.

Reduction of Inflammation and Collagenase Production in Experimentally Induced Adjuvant Arthritis

Adjuvant arthritis (AA) was treated orally in rats with 13-cis-retinoic acid to determine its effects on the production of collagenase and prostaglandin E2 (PGE2) by adherent cells taken from the inflamed ankle joints of these rats in terms of increased or decreased synthesis in monolayer culture.

Adjuvant arthritis, which shares a number of features with rheumatoid arthritis, is characterized by an initial inflammatory response in the adjuvant-injected paw and a systemic response consisting of a chronic inflammatory response in both the injected and contralateral paw. Treatment with 13-cis-retinoic acid suppressed the development of AA in both the initial and later manifestations of the condition with the greater effect seen with the later manifestation involving both the ipsi- and contralateral paw. 13-cis-retinoic acid was also effective in the treatment of established AA, as indicated by reduced inflammation.

Less collagenase was secreted from cell from rats with AA treated retinoic acid while the PGE2 concentrations in these cultures were similar for all arthritic groups whether treated with vehicle only or 13-cis-retinoic acid. The data were consistent with earlier studies concerning the effects of 13-cis-retinoic acid on collagenase and PGE2 production of primary cultures of rheumatoid synovial cells in which the concentration of collagenase was lowered to undetectable levels, while the concentration of PGE2 decreased only slightly.

The successful use of retinoids in the treatment of this proliferative disorder demonstrates a new application of these compounds with the hope that 13-cis-retinoic acid or other related retinoids will eventually be helpful in treating rheumatoid arthritis.

The mechanism by which 13-cis-retinoic acid acts to decrease the inflammation and collagenase production associated with AA is not clear and needs further study. The possible involvement of PGE2 deserves consideration since, in addition to being an important mediator of inflammation, it may also modulate cell mediated immunity. Retinoids have been shown to both increase and decrease PGE2 synthesis, depending on cell type, the specific retinoid and its concentration and further study is needed. Other separate studies will continue investigation of the kinetics of arachidonic acid turnover in human synovial fibroblasts to determine the mechanism of prostaglandin stimulation by a number of stimula factors including an interleukin-1 like factor. In addition, this kinetic model system will be used to study the mechanisms of inhibition of prostaglandin synthesis by glucocorticoids. Reasons need to be provided for the disparate effects of treatment with 13-cis-retinoic acid on the severity of the induced arthritis in the two models tested. The ultimate goal is to develop a strong rationale for treating human rheumatoid arthritis.

Utilization of Radioisotopes for Synovectomy in Patients with Rheumatoid Arthritis

The major cause of pain in rheumatoid arthritis is destruction of arthrodial or synovial joints. The origin of pain and ultimate joint destruction lies in the inflammatory response in the synovial lining of affected joints. Continued inflammation not only perpetuates pain and loss of motion but ultimately leads to enzymatic destruction of the articular cartilage. The central role of the inflamed synovium and its population of cells in the known sequence of events leading to joint destruction suggests that early medical control of the synovial inflammatory process or ablation of the inflamed synovium will either prevent or delay joint destruction.

An alternative to surgical synovectomy with its attendant discomfort and high recurrence rate is the use of radioisotopes by intra-articular administration to destroy diseased synovium. An animal model of immune arthritis has been used to evaluate various radiopharmaceuticals and methods of packaging these isotopes to avoid systemic spills, and thus produce an effective synovectomy.

Of five potentially useful radioisotopes, the use of 165 dysprosium attached to a ferric hydroxide microaggregate has been determined to be the most promising. The appropriate dose that will produce synovectomy without damaging articular chondrocytes has been achieved.

Animal studies have been extended to humans with symmetrical rheumatoid involvement of the knees. The effectiveness of the resulting synovectomy has been evaluated by both clinical observations and objective scanning. Improvement in articular function and objective parameters in 80 percent of patients treated for periods of twelve months have been reported.

Clinical studies with dysprosium and additional research towards production of new isotopes and new particle systems, to extend and improve these preliminary results, will continue.

MUSCULOSKELETAL DISEASES RESEARCH AND TRAINING PROGRAMS

The Musculoskeletal Diseases Program provides research support for basic and clinical studies on the various components of the musculoskeletal system in normal and diseased conditions. The program utilizes funding mechanisms such as regular research grants, career development awards, training grants and contracts.

The Program and its staff have organized and supported several workshops and conferences in FY 84. An NIH Consensus Development Conference on Osteoporosis was held in April, 1984. There was great public interest in the topic as evidenced by the large number of attendees (over 750) and the broad coverage by news media. Highlighted recommendations for prevention and treatment were estrogen replacement in at-risk postmemopausal women, calcium supplementation, physiologic levels of vitamin D, and mild excercise (such as walking). In the fall of 1984, a workshop will be sponsored on mineralized tissues. Leading international scientists will discuss the complex process of bone cells laying down a matrix that is subsequently mineralized. A state-of-the-art book will be prepared from the workshop manuscripts.

Several projects that were in progress during FY 83 were completed this year. A summary of the workshop on Local Factors Influencing Bone Formation was published in the Journal of Calcified Tissues. The workshop on Bone Allograft Transplantation was summarized in an article to be published in Clinical Orthopaedics and Related Research. The final report on the evaluation of the Musculoskeletal Diseases Program has been printed and is being distributed to researchers in the field.

Local Factors of Regulation of Bone Formation

Human skeletal growth factor is a high molecular weight protein that may control the linkage and balance between the formation of bone and its continuous resorption. It has shown the capacity to couple resorption and formation. This substance interacts positively with calcium regulating hormones (pTH and $1,25(OH)_2$ Vitamin D), is a unique mitogen, and acts on cells other than bone.

Other local factors include low molecular weight proteins such as bone derived growth factor (isolated from incubation media of organ cultured bones) and macrophage derived growth factor (obtained from conditional medium bathing organ cultured microphages). The higher molecular weight factors stimulate calvarial DNA synthesis. While the lower molecular weight factors stimulate bone collagen. Another protein, osteonectin, may have a role in attaching bone mineral to the matrix. It stimulates synthesis of collagen and other proteins and increases alkaline phosphatase activity.

Defects and imbalances in the dynamic processes of bone remodeling can lead to serious disorders such as osteoporosis, osteomalacia, and Paget's disease. Advances such as these in our understanding of the regulation of bone formation by local factors are an important key to future advances in bone disease research. Fracture repair and surgical intervention may also be enhanced by improved knowledge of the controlling factors.

Growth Plate Physiology

Growth plate disorders from genetic defects or traumatic disruption can lead to serious developmental problems. Treatment must be based on an understanding of normal development and mineralization of this specialized portion of bone. The systemic regulation by various growth factors and their interactions is a key to current and future therapy.

Animal studies with bovine growth plate have shown that somatomedin-C binding is 3-5 fold greater in the proliferative zone than in other zones. It also appears that the effect of somatomedin may be different at different stages of cellular maturation. One of the structural components of epiphyseal matrix, glycosaminoglycin, was shown to be stimulated with somatomedin and inhibited by cortisol. There was a lesser effect on collagen and DNA synthesis. Deficienc of manganese and zinc in chick models caused significant growth retardation. In one growth plate disorder (tibial dyschondroplasia) the site of defect was found to be incompletely developed hypertrophic cells that did not mineralize or properly resorb the matrix.

The research is continuing on normal physiology and disorders of the growth plate. Electron microprobe analysis is underway to determine the concentration of elements in the cells and matrix of all zones. The current technology in serum free cultures will allow rapid advances in this field.

Collagen Defects in Osteogenesis Imperfecta

Studies are in progress on the genetic, molecular and biochemical aspects of the defects in patients with osteogenesis imperfecta (OI). Osteogenesis imperfecta is a heterogeneous genetic disorder producing various forms and degrees of fragile bone. In both OI type II A and II B defective regulation appears to occur. The pathogenesis of II A seems to be insufficient accumulation of osteoid for normal bone formation, whereas II B may be caused by incorporation (and later proteolytic removal) of a defective collagen, leading to a "moth-eaten" defective osteoid. A specific finding in OI Type II A cell lines is a biphasic, non-linear rate of synthesis and secretion of procollagen. In a genetically dominant form, there was an abnormal ratio of alpha 1 to alpha 2 collagen chains, with accumulated material in the rough endoplasmic reticulum. The excess material is probably related to degraded products of incompletely formed Type I collagen. In one mild form of the disease there appears to be a low content of alpha 1 (I) m RNA, suggesting the presence of a non-functional allele of the alpha 1 (I) gene. In two cases there was a direct relationship between the gene function and decrease in total collagen synthesis.

In the past, treatments for the rare, but severely disabling conditions of OI have been based on symptomatic relief from fracturing. The underlying defects are becoming better understood. In the future, patient management may be based on replacing the specific defective biochemical component or gene responsible for production of collagen and bone.

Action of Vitamin D Metabolites

In patients with cross-linked hypophosphatemic osteomalacia (rickets) the serum 1,25 $(OH)_2$ Vitamin D levels are about the same as age and growth-rate matched normals. When treated with Vitamin D the metabolite levels are reduced. This implies a reduced capacity for metabolism relative to the hypophosphatemic stimulus (most likely due to defective hydroxylase activity). Serum concentrations of 1,25 $(OH)_2$ Vitamin D are often higher during growth than in adults. It appears due to a loose regulation by hydroxylase during development. In a hypercalcemia disorder in infants, Williams syndrome, the final hydroxylation is defective.

Many studies are progressing with animal models. In Vitamin D deficient chicks there is at least 70% more non-mineralized collagen than in controls. Also the final metabolism to 1,25 $(OH)_2$ Vitamin D and 24,25 $(OH)_2$ Vitamin D can occur in all tissues related to mineral homeostasis and not just the kidney. This extra-renal metabolism may have physiologic significance.

Bone Infection

Infection in bone is a serious problem that is of growing prevalence due to increased implantation of orthopedic devices. The use of prophylactic antibiotics in bone cement is common but not based on basic and clinical studies.

Antibiotics that are commonly used in bone infections can freely penetrate the marrow cavity provided that there are not physical barriers. The distribution is not uniform throughout the marrow tissue. Antibiotics seldom enter bone canaliculi and never penetrate the mineralized tissue. However, this is not of concern since the 0.1 micron diameter canaliculi are too small for bacteria to enter. Tobramycin crosses the capillary membrane of normal and osteomyelitic bone and achieves bactericidal concentrations.

Several advances have been made in characterizing the nature of resistant bacteria. Some bacteria on prostheses formed adherent microcolonies that surrounded themselves with an amorphous matrix. Also an intercellular persistance of organisms, such as S. aureus, may be the cause of recurrent infection. This may arise from a walling off of the infected area that requires intense resorption before white cells can reach the bacteria. Research with direct clinical application in this area is anticipated. Several animal models of osteomyelitis are available to reproducibly develop bone infection.

Factors Influencing Osteoarthritis

Osteoarthritis is the most prevalent motion-limiting disorder. The etiology, especially the reluctance of cartilage to repair itself, is not well understood.

One possible influencing factor that has been studied is aspirin. In an exper ment, the anterior cruciate ligament of dogs was torn to simulate osteoarthritis changes. Dogs fed aspirin were compared with controls that were not. Those fed asprin showed a significant reduction in proteoglycan synthesis, but not a corresponding increased degradation of proteoglycan. These results indicate that injured knee joints show greater susceptibility to the effects of aspirin, possibly because the depletion of proteogycans in the matrix leads to changes in permeability.

Other advances in understanding of factors relating to osteoarthritis are in the area of mechanical factors. In several animal models with increased impulsive loading and walking on hard surfaces, subchondral bone microfractures and subsequent stiffening have been shown to precede damage to the cartilage. An in-vitro experiment has shown that stiffening of the subchondral bone with methyl methacrylate (bone cement) significantly increased articular cartilage wear.

SKIN DISEASES PROGRAM

The Skin Diseases Program of the NIADDK provides research support for basic and clinical studies of the skin in normal and diseased states. Areas of emphasis include: psoriasis and disorders of keratinizing tissues; vitiligo and other disorders of pigmentation; photobiology, photo-allergy; and photo-toxic reactions; metabolic studies of skin, including effects of hormones and interaction with enzymes; immunologically mediated cutaneous disorders, including atopic dermatitis, eczema, contact dermatitis, and vasculitis; bullous diseases of the skin, including pemphigus, pemphigoid, dermatitis herpetiformis, and epidermolysis bullosa; acne and physiologic activity of the sebaceous gland; disorders of hair growth, including alopecia areata; and cutaneous manifestations of connective tissue disease disorders, including lupus erythematosus, scleroderma, and pseudoxanthoma elasticum.

The Skin Diseases Program utilizes many funding mechanisms, including research grants, career development awards, Clinical Investigator Awards, Physician Scientist Awards, New Investigator Research Awards, training grants, cooperative agreements and contracts.

The Skin Diseases Program contributed significant support to the 33rd Annual Symposium on the Biology of Skin, on the subject of "Cutaneous Oncogenic Viruses." At this meeting, strong emphasis was placed on the papilloma and herpes families of viruses, both of which have members capable of transforming cells in vitro. The proceedings of this symposium were published in the July 1984 issue of the Journal of Investigative Dermatology.

The Skin Diseases Program also contributed to the support of the New York Academy of Sciences' "International Conference on Intermediate Filaments." One day of this three day meeting was devoted to keratin, the intermediate filament characteristic of skin. The proceedings of this meeting will be published in book form.

Structure and Function of Keratins in Normal and Diseased Skin

By the application of immunologic, molecular genetic, and cell culture techniques much has been learned about the structure and function of the epidermal keratins and their expression during differentiation, embryonic skin development and in certain disease states.

The keratins comprise a family of 10 to 20 related polypeptides of molecular weight 40 to 70 kd that form 8 nm cytoplasmic filaments in most vertebrate epithelia. These keratins can be subdivided into two distinct types based on (1) the ability of their corresponding messenger RNAs to crosshybridize with two different cloned keratin DNAs, and (2) the ability of individual polypeptides to cross-react with two different antikeratin antibodies. Type I keratins are acidic and generally smaller, usually with a molecular weight of 40 to 52 kd, whereas, Type II keratins are basic and usually larger in size, their molecular weight being between 53 and 67 kd. Each epithelial cell type expresses only 2 to 6 polypeptides of the total keratin family, and at least one member of each keratin type is always made in any epethelial cell.

Recently, Fuchs and associates, supported by this Institute, have sequenced the cloned DNAs corresponding to a Type I 50 kd and a Type II 56 kd keratin from cultured human epidermal cells. The predicted amino acid sequences of these two keratins show only 30% homology; yet both sequences are compatible with a common secondary structure. The central aproximately 300 residue long region consist of four largely alpha-helical domains demarcated by three small, conserved, non-helical segments for which beta-turns are predicted with high probability. The amino and carboxy terminal regions are clearly non-helical and both are greater in length for the larger Type II keratin. The carboxy terminus of the Type I keratin and the amino and carboxy termini of the Type II keratin contain unusually glycine-rich repeat sequences. These findings have been confirmed by Freedberg et al., supported by this Institute, and by Steinert et al., supported intramurally in the Cancer Institute.

Studying mammalian epidermal keratin filaments in vitro, Steinert has recently demonstrated that these filaments are larger than previously recognized. The filaments are actually 15 to 16 nm wide rather than the previously accepted value of 8 to 10 nm. This extra width is attributed to a lower mass region surrounding the denser core of the keratin filaments. This low mass region apparently consists of the glycine-rich non alpha helical portions which protrude laterally from the central core. In the past, only the central core has been measured. These newly described glycine-rich portions may provide the mechanism by which keratin filaments interact side by side with one another to form the densely adherent close packed regular network in normal epidermis.

Dale, Holbrook, and coworkers supported by this Institute have investigated the appearance of various keratin proteins during development of human fetal epidermis, utilizing numerous biochemical and immunologic techniques, including monoclonal antibodies. They have demonstrated that very small 40 and 52 kd keratins are present early in development and decrease as development progresses. These keratins can be demonstrated in the cells of basal and intermediate epithelial cells and especially in the periderm cells until 11 weeks of development, but at later times are found only in the basal cell layer and the periderm. The larger 56 and 67 kd keratins appear and increase dramatically between 11 and 14 weeks gestational age. These keratins can be demonstrated in the intermediate cell layers at 12 weeks gestational age and older, and in the keratinized epithelium at 24 weeks gestational age. Keratinization is present at 14 weeks around hair canals, but otherwise is not present in the epidermis until 24 weeks gestational age. These investigators have also studied the appearance o a protein called filaggrin, which functions in the assembly of keratin filaments in the formation of stratum corneum. Filaggrin appears at 14 weeks gestational age in association with the early keratinized hair canals, and appears later in development in the stratum corneum when that structure keratinizes at 24 weeks gestational age. Thus, by 14 weeks gestational age all of the keratin proteins of the epidermis are present within the skin of the fetus. This would indicate that some disorders of keratinization in which structural proteins are altered may be diagnosable by their presence in the amniotic fluid as early as 16 weeks gestational age, and be ascertained by the simpler procedure of amniocentesis at this time rather than by the more complicated procedure of fetoscopy and fetal skin biopsy at a later stage in development.

Several levels of control mechanisms for the process of keratinization have been demonstrated, including post-translational modification, an example of which is the assembly of the keratin filaments under the control of filaggrin; gene control associated with the degree of differentiation, such as the synthesis of the higher molecular weight keratins by more differentiated epidermal cells; and the effect of small molecular weight molecules such as vitamins and hormones, including vitamin A and estrogens, which control the synthesis of the various keratins in certain tissues under certain conditions in both normal and abnormal states. This detailed knowledge may be applied to altering skin both in adults and even in utero. The defining of the small molecular weight modulaters will allow treatment of skin diseases even including treatment *in utero*. Conditions such as ichthyosis may well be targets for such treatment; it has been demonstrated in other studies by Dale *et al*. that filaggrin is found in excessive amounts in one form of ichthyosis (epidermolytic hyperkeratosis). Prenatal diagnosis will be greatly advanced by our knowledge of the normal appearance o the different forms of keratin and the stage of fetal development at which the abnormal keratins may be noted in a number of genetic diseases. It is now also possible to analyze for these abnormal keratins by the presence of their messenger RNA before the keratin itself is phenotypically visible in the epidermis. Thus, these basic advances in the biology of the keratins has the potential for opening many new avenues in pre- and post-natal diagnosis and treatment of numerous skin diseases in which there are abnormalities of keratinization.

MULTIPURPOSE ARTHRITIS CENTER PROGRAM

Center Research Projects

Inherent in the concept of a Multipurpose Arthritis Center is a strong research component. Center grant support is intended to complement traditional research grant support in a given institution to establish related special projects and stimulate the development of new research projects. Consequently, each potential Multipurpose Arthritis Center is expected to be receiving research grant support for basic and/or clinical biomedical research related to rheumatic diseases as a prerequisite for a center grant award. Each center, therefore, possesses a substantial research base to examine the cause of rheumatic diseases, and, often to study the means of improving their diagnosis and treatment.

Biomedical research projects supported by center grant funds are almost exclusively developmental and feasibility studies. These studies are designed to encourage investigators to explore interdisciplinary and highly innovative scientific approaches which may later form the basis of applications for traditional research grant awards from the National Institutes of Health or other agencies.

Investigators at the Case Western University Arthritis Center located in Cleveland, for example, last year began studying a substance known as C-reactive protein (CRP), a molecule synthesized by the liver in response to tissue injury, with a goal to understanding its role and biological significance in acute inflammation. Their first efforts are focused on determining the mechanism of CRP induction and how its biosynthesis is regulated. To do this, molecular genetic techniques are being used to examine how messenger RNA (mRNA) is formed both in normal rabbit liver and from rabbit livers 24 hours after they have been subjected to an inflammatory stimulus. Initial results have shown that even after such a stimulus an increased amount of messenger RNA fails to develop. This is a surprising result since theory would have predicted more mRNA to be present as a consequence of inflammation. In addition, in related studies the catabolic rate of CRP was found to be quite rapid and independent of serum CRP concentration. All these findings indicate that the liver plays a crucial role in the regulation of CRP synthesis and catabolism during the inflammatory response. Future experiments will involve analyzing CRP mRNA immunochemically and studying the mechanisms of hepatic uptake of extracellular CRP.

Several studies at the Centers are concerned with orthopedics. At Northwestern University's new Multipurpose Arthritis Center in Chicago, for example, a project is underway to define the mechanical properties of cancellous bone in rheumatoid and osteoarthritis patients undergoing total knee arthroplasty (joint replacement surgery). The investigators hypothesize that mechanical properties of bone, and the subsequent bone-cement interface, differ in osteoarthritic and rheumatoid patients. If this conjecture is correct then prosthetic design requirements might therefore also differ for these two groups of patients.

Another important project related to bone has just been initiated by the Arthritis Center at the University of Michigan in Ann Arbor where investigators are evaluating the capacity of electron microscopic imaging techniques to detect pathologic changes in articular cartilage and bone in patients with osteoarthritis. Studies are being made with: sonographic imaging; bone scans to detect microfractures in remodelling bone; and nuclear magnetic resonance to define characteristics in experimental models of osteoarthritis. The reason for making these studies is that, unlike osteoporosis where one can monitor loss of bone long before overt disease occurs, osteoarthritis, which involves breakdown of cartilage with almost no changes in bone in early stages, has no way at the present time of being detected noninvasively. Advanced disease is often present by the time there are joint changes apparent by x-ray. If there were non-toxic and non-invasive scanning methods available, it might be possible to select patients who could benefit from prophylactic management. This study represents an important opportunity to examine one of the most important questions in clinical research related to osteoarthritis, namely, how to assess early cartilage damage by noninvasive techniques.

At the Arthritis Center at Brigham and Women's Hospital in Boston investigators at the Arthritis Center are developing a method to evaluate the success of total knee replacement surgery by gait analysis. The orthopedic surgeons involved hypothesize that lower limb function following total knee replacement relates to factors, such as muscle function, other than the prosthesis itself. A group o patients who have had knee replacements will be studied with respect to a number of parameters, including three-dimensional gait analysis and energy consumption as measured by oxygen uptake. These studies represent the utilization of a well established technology, previously used for children with cerebral palsy, to study a problem of enormous economic and social significance in this country.

A number of projects in the Arthritis Centers deal with rheumatoid arthritis synovial (joint lining) tissue. As an example, in a project underway at the University of Alabama in Birmingham, Center scientists have noted the substantial evidence of local antibody formation at synovial sites in rheumatoid arthritis and propose to characterize B cells (antibody-producing cells) in synovial tissue in terms of immunoglobulin surface markers. The investigators will determine whether there is restricted diversity in the type of those cells present at the inflammatory site using hybridoma technology. Attempts will also be made to clone cells from the synovial sites to assay both their antigenic characteristics and the antigenic specificities of the antibody they produce. It is hoped that the information gained from this analysis will be useful in providing an explanation of how the inflammatory response progresses in rheumatoid arthritis.

In a related study at the University of California in San Francisco the techniques of molecular biology are being applied to search for a cell or cell line in rheumatoid synovium which exhibits changes in chromosome structure (oncogene expression) that are usually asosciated with cellular transformation (a process which is also observed in malignant cells). If cells at chronic inflammatory sites are found to have such characteristics, fundamental questions would be raised about the functional significance of oncogene expression. That is, if oncogenes are found to be "turned on" in some non-malignant conditions, such as rheumatoid arthritis, it would be significant for the study of genetic control mechanisms in general and possibly lead to new approaches in the treatment of rheumatoid arthritis.

Center Education Projects

Another integral aspect of the Multipurpose Arthritis Centers program involves educational activities designed to facilitate and increase the education of all types of health professionals. As an example, the University of Connecticut's Multipurpose Arthritis Center is continuing to develop and evaluate computer-based education protocols for physicians and patients on rheumatoid arthritis, gout, and systemic lupus erythematosus. The course includes sections on treatment, medication, exercise, joint protection, the use of adaptive equipment and splints, patient compliance, psychosocial issues, relaxation techniques, and the use of non-prescribed therapies. After the series of lessons are completely developed, they will be transferred to microcomputers and be available nationally to anyone with access to a personal computer.

At the University of North Carolina Arthritis Center in Chapel Hill a patient education project is being conducted with a goal of systematically developing a way for patient educators and other health professionals to identify effective interventions to increase the rheumatoid arthritis patient's compliance with his or her treatment regimen. Each patient is being interviewed to identify the physicial, psychological, social, and environmental inhibitors and facilitators of compliant behavior. The information obtained is being used to plan feasible and potentially effective interventions to increase compliance. When complete, this project will contribute to the field of arthritis patient education by providing patient educators and other health professionals with a procedures manual containing guidelines for a psychosocial diagnosis to identify reasons for noncompliance and assess patients' needs for appropriate education or referral sources.

Another patient education program, being conducted at the Arthritis Center at Northwestern University in Chicago, will develop, implement, and evaluate an education program to teach aerobic-like exercise to rheumatoid arthritis patients and individuals with osteoarthritis. A number of variables will be studied, including: physiological variables, such as joint inflammation, muscle strength, and functional capacity; and psychological variables, such as self regard, level of wellness, and perceived level of pain. Besides attempting to determine if this educational and exercise program can be effective in improving the physiological and psychological well-being of arthritis patients, the investigators hope to learn if the program works better for some types of arthritis patients than for others and if its effectiveness is modified by the patient's level of education or motivation level. This study should help to shed light on the efficacy of aerobic-like exercise for those with arthritis.

At Boston's Brigham and Women's Hospital Arthritis Center an educational program has begun to evaluate how low back injuries can be prevented at the worksite. The aims of the study are: to reduce the incidence of lifting-related back injuries; and to reduce the average duration and total number of days lost from work due to back pain and lifting-related injuries. Subjects will include approximately 4500 Boston postal workers and supervisors who will receive either no intervention or education about back injuries along with periodic follow-ups. In addition, physicial therapists will monitor actual lifting practices. Variables to be measured in the study include back pain, psychological well-being, depression, cardiovascular fitness, alcohol consumption, and smoking history. If successful, this study could have important economic implications by reducing the amount of work-related back injury and disability.

Center Community/Health Services Research Projects

Community and health services research projects conducted by the Multipurpose Arthritis Centers deal with a diverse range of activities including, for example, health economics and the costs of care for persons with rheumatic diseases, measurement of functional outcome variables and disability, epidemiology of rheumatic diseases, the effect of chronic illness on the quality of life for both adults and children, and the assembly storage, retrieval, and analysis of data dealing with populations of persons with rheumatic diseases.

A study underway at Case Western University's Multipurpose Arthritis Center is designed to explore the problems that adults with rheumatoid arthritis and children with juvenile rheumatoid arthritis have in obtaining their rights and benefits under current disability and handicap laws. The nature of these diseases makes it difficult at times to establish a definite clinical diagnosis. In addition, characterisitic periods of flares and remissions may make it more difficult for these patients to obtain their rights and entitlements. So far

100 adult and 200 juvenile rheumatoid arthritis patients have been surveyed to identify the types and frequency of handicap and disability law-related proble experienced by these patients. The information obtained from these interviews will be analyzed by legal experts and correlated with federal and state laws and regulations. After this analysis is completed two sets of legal materials, one designed for patients and a second designed for health care and legal professionals, will be written by experts in community education and by lawyers specializing in problems of the chronically ill, handicapped, and disabled.

In order better to manage the care of patients with rheumatic diseases, especially in areas which may not have the services of a trained rheumatologist, the Arthritis Center at the University of Missouri in Columbia is developing a computerized rheumatology consultant system. The clinical situation is that of the physician who considers that he or she may require a rheumatology consultation for a new or an existing patient. The management model will determine whether there is a rhematologic problem present, how the patient work-up should be structured in terms of physical examinations performed, whether possible referral is required, or which of the therapeutic management plans within the computer would be most appropriate. The system so far contains 26 disease states, including rheumatoid arthritis, systemic lupus erythematosus, progressive systemic sclerosis, gout, infection-induced arthritides, juvenile rheumatoid arthritis, and osteoarthritis. It has been retrospectively tested against 443 cases with an overall accuracy of 94%. The computer will refuse to make a diagnosis for items which are not in its knowledge base and also identifies which treatment situations are not safely undertaken by the non-expert, with or without the computer consultant. After the model is completely tested, it will be adapted to a microcomputer for distribution around the country.

As part of a collaborative investigation with the Tecumseh Community Health Study (a longitudinal prospective analysis of a number of diseases in a Michigan community) the Arthritis Center at the University of Michigan in Ann Arbor is beginning to analyze the epidemiology and risk factors associated with osteoarthritis. In addition to re-reading of the initial radiographs of hands and wrists taken approximately 20 years ago, data concerning weight, socioeconomic status, occupational activity, joint symptoms, and biochemical parameters will also be analyzed. This study is potentially useful if predictive factors in disease development can be identified.

Another epidemiologic study, being conducted by Boston University's Arthritis Center, will investigate the association between the use of oral contraceptives by women between the ages of 15 and 44 and the development of rheumatoid arthritis. This study, which will analyze data on over 100,000 women, will provide more detailed information on oral contraceptive use and the development of rheumatoid arthritis than has been present in the past. It will examine if the protective effect depends on the estrogen or progesterone content of the pill. The effect of length of oral contraceptive use and dose of estrogen used will also be explored. If the protective effect of oral contraceptives is confirmed, this study will contribute to the understanding of the pathogenesis of rheumatoid arthritis, and in addition, will yield information on the relation of sex steroids and autoimmune disease.

Core Units

During the past year, a number of core units were in operation at several of the arthritis centers. These common-use facilities are designed to increase the effectiveness and efficiency of each center's activities. For biomedical research, there are core units for tissue culture, connective tissue matabolism, immunology, histocompatibility testing, and research involving hybridomas, to name just a few. Core units in other areas are concerned with biostatistics, evaluation methodologies, educational activities and epidemiology.

The Center located at Indiana University in Indianapolis has had two core units in operation during the past year. The first, dealing with immunology, has functioned as a centralized facility in which specialized tests of human immune function can be performed, including assays for immune complexes, assays of in vitro antibody synthesis, and assessment of T and B lymphocytes in peripheral blood. The other core, concerned with connective tissue biochemistry, has been designed to separate and purify proteins and other complex compounds derived from a variety of soft tissues. Thus, this core has developed with capability to separate substances by various types of electrophoresis and analyze the result by specially designed computer software.

A core unit at the University of Michigan's Center in Ann Arbor is active in providing investigators with tissue culture support for a number of diverse projects including: the isolation and characterization of proteins important in activating human connective tissue cells; a study showing that fibroblasts from scleroderma patients have increased rates of hyaluronic acid synthesis; and identification of a new connective tissue activator factor in human urine.

An immunoassay and immunoreagent development core unit at the University of North Carolina in Chapel Hill is responsible for: maintaining a 'library' of human antibodies used by a number of investigators involved in the Center; preparing antibodies to human proteins; and developing methods for conducting immunologic assays which will be available to Center investigators on a routine basis.

A cell culture core operating at the Arthritis Center at Case Western University in Cleveland provides investigators with cell and tissue culture laboratory facilities for morphologic and biochemical studies such as those dealing with enzyme purification, proteoglycan and collagen biosynthesis, and in vitro behavior of mammalian chondrocytes and cartilage metabolism in aging, the role of cell-mediated immunity in rheumatoid arthritis and osteoarthritis, and studies of the role of various hormones on the pathophysiology of cartilage and osteoarthritis. At the same center an education and evaluation core unit provides a mechanism for evaluative review and prospective analyses of ongoing center projects and provides recommendations for new projects and activities in which the center can become involved. The members of the core review protocol design, data collection methods, instrument development, statistical analysis, and provide guidance to the individual project investigators in process, impact, and outcome evaluation.

Similar core units dealing with statistics and evaluation are in operation at the Arthritis Centers at the University of Connecticut, the University of North Carolina, Boston University, Stanford University, and Northwestern University.

These cores provide expertise in research design and planning, data collection data base management, computer programming, data analysis, and act as a technic resource regarding statistics.

The Arthritis Center located at Brigham and Women's Hospital in Boston has several cores, including: an immunogenetics core, to support the evaluation of genetic factors in rheumatic disease by performing assays to detect the major histocompatibility antigens on patients with various forms of arthritis; a hybridoma core, to produce monoclonal antibodies for projects dealing with such areas as complement production, proteoglycan synthesis, and systemic lupus erythematosus; a morphology core, which will perform a wide range of laboratory techniques such as scanning and electron transmission microscopy, immunocytochemistry, and x-ray analysis; and a biometry core, which will provide statistical and computational support for many Center projects.

SPECIAL PROGRAMS IN ARTHRITIS AND RELATED DISEASES

The following important activities are being developed to address certain specific features of the National Arthritis Acts of 1974 and 1976, and several recommendations of the Arthritis Plan submitted to the Congress in April 1976 by the National Commission on Arthritis and Related Musculoskeletal Diseases.

Education Programs

Creation of the Education Programs Office has brought the challenge of communication of arthritis information into sharp focus and resulted in a higher public recognition of the need for coordination of various efforts. Beyond mere coordination, however, lies the need to ensure development of practical, useful materials to aid arthritis health professionals. The primary goal of the Education Programs Office is the maximum use of the tools of communication to inform and educate arthritis health professionals about arthritis. Significant progress is being made towards stimulating and assisting development of public, patient and professional education as essential elements in achieving optimal treatment and management of arthritis. The Office also serves as an important instrument for bringing together government, the private sector, the professions, and others in a cooperative educational effort to combat arthritis.

Arthritis Information Clearinghouse

A major component of the Education Program Office is the Arthritis Information Clearinghouse (AIC). Established by the NIADDK following authorization by the National Arthritis Act and recommendation by the National Commission on Arthritis and Related Musculoskeletal Diseases, the Clearinghouse aims to improve the lines of communication among health professionals serving arthritis patients. Major functions of the Clearinghouse are to collect, screen, store, and disseminate information about educational materials and programs on the rheumatic diseases. In addition to this service to providers, the Clearinghouse seeks to acquire a better understanding of information needs, especially the unmet ones, of arthritis patients, and to communicate these needs to the field.

In serving as a "broker" to foster the flow of arthritis information, thus helping users to locate and select educational materials, the Clearinghouse refers clients to the appropriate developer or source rather than acting as a distributor of the materials itself.

Data Base of Educational Materials

During this past fiscal year, the Clearinghouse consolidated its "holdings" in the National Combined Health Information Database (CHID). The CHID is the outgrowth of a meeting two years ago by members of seven federally supported health information and health education clearinghouse to discuss the possibility of combining their individual bibliographic files into an online database to avoid duplicative efforts, to share information and expertise, and to increase ease of access for users. The database, now a reality as a private file on BRS, presently includes the files of the Arthritis Information Clearinghouse, the National Diabetes Information Clearinghouse, and the Center for Health Promotion and Education. Three other clearinghouses are considering joining, and are presently making arrangements to reformat their records.

The general information in the new database does not duplicate MEDLARS but rather focuses on somewhat different areas: health resources and patient education materials (particularly so-called "fugitive" literature), patient and professional educational programs and curricula, and evaluation material (in the specific category of information that each clearinghouse represents). Many of those materials are not available through libraries, on-line databases, or other sources considered traditional. "Fugitive literature" is so named because it is not published in the traditional medical journals but rather comes from hospitals, voluntary health organizations, pharmaceutical firms, and specialized health information publishers. The health professionals who look for these materials usually direct patient education or are involved in developing patient education programs. They need such materials for patient education documents such as teaching manuals, program design instructions, and evaluation materials, and for information about new interventions or procedures.

The new database, consisting of the files of the three current participants, became available this past fiscal year and contains more than 10,000 records. As of the end of this fiscal year, the arthritis file on BRS contains 3,593 records, and regular updates on new materials to the file continue to be made.

Biblio-Profiles

Biblio-profiles, a new kind of publication for health professionals, are now available from the Arthritis Information Clearinghouse. A biblio-profile is a brief yet comprehensive state-of-the-art report (the profile), followed by a selected bibliography (the biblio). The intent of the biblio-profile is to provide an overview of a topic, indicate its current importance to the field, and point to areas where little is known or where gaps in knowledge exist. The Clearinghouse and its Advisory Group select the topics for this new series, and subject experts prepare them.

Several new biblio-profiles were prepared during this reporting period and include The Role of Primary Care Physicians and Allied Health Professionals in the Care of the Arthritis Patient, by Eric P. Gall, M.D.; Update on Corticosteroid Therapy in Rheumatic Diseases, Biblio-Profile no. 3, by Edward V. Lally, M.D., and Stephen R. Kaplan, M.D.; and Physician-Patient Communication, by Michele Boutaugh, R.N., MPH.

Other Products and Services

Five annotated bibliographies and eight reference sheets were completed by the Clearinghouse during the year. Two issues of the Clearinghouse newsletter were prepared and distributed. Each of the bibliographies and reference sheets has been reviewed by authorities in the field and covered:

Ankylosing Spondylitis
Diagnostic Radiology in the Rheumatic Diseases
Infectious Arthritis
Juvenile Arthritis
Lupus: Professional Education Materials
Biofeedback
Diet and Arthritis
Drug Information for Patients
Gout: Patient Education Materials
Lyme Disease
Multipurpose Arthritis Centers
Scleroderma: Patient Education Materials
Splints

Some 1,447 names of individuals or organizations were added to the Clearinghouse mailing list, bringing to total to 6,782 names. The mailing list has been coded to make the identification of specific professionals--physicians, librarians, health educators, and pharmacists--possible.

Cooperative Efforts

The Education Program Officer and the Clearinghouse staff have evolved a close working relationship with the Subcommittee on Education and Training of the National Arthritis Advisory Board, providing logistical and technical support whenever possible. The Clearinghouse has also continued its efforts to collect information on community and institutional patient education and in-service training programs within the Multipurpose Arthritis Centers (MAC). The Clearinghouse continued to act as a depository for MAC reports, reprints, and studies. Curricula and program descriptions of patient education or in-service training classes are being collected, and materials developed such as syllabi, resource lists and supplementary materials are being sought.

As part of its outreach program, periodic memoranda are published to alert users and health professional constitutents as to new developments. In addition, a Clearinghouse exhibit has been sent to meetings and conferences, including annual meetings of the American Rheumatism Association , the Medical Library Association, and regional arthritis-related meetings.

Advisory Group

An advisory group comprised of eleven rheumatologists, educators, library scientists and other health professionals, has continued to help the Clearinghouse in effective planning and accomplishment of goals. The group, meeting twice yearly and in periodic telephone and letter communication, provides recommendations to the Clearinghouse project officer and staff.

EPIDEMIOLOGY DISEASES PROGRAM

The Epidemiology Program provides an administrative core for efforts to encourage epidemiologic research in the fields of arthritis and musculoskeletal diseases. Epidemiologic studies of arthritis and musculoskeletal diseases contribute knowledge related to three concerns: assessing the community disease and disability burden, studying the natural history of disease, investigating disease etiologies and identifying risk factors. Currently the portfolio of epidemiology grants includes regular research grants, training grants, an individual fellowship, new and clinical investigator awards and contracts, at a cost of approximately one million dollars. In addition, there are epidemiology projects within other grants, such as the Multipurpose Arthritis Centers.

The number of epidemiology investigator initiated applications has continued to grow during the past year, although the rate of fundable applications remains low. A positive trend has been noted in the increasing volume of successful amended applications.

Arthritis Data Work Group

The Arthritis Data Work Group has been formed in response to an ongoing need for reliable data on the various forms of arthritis. Over the years NIADDK, the National Arthritis Advisory Board (NAAB) and the Arthritis Foundation (AF) have been the frequent recipients of requests for data on arthritis. For some of these requests, usable data do not exist, while for others different overlapping sources of data are available, but must be used with caution. In order to address this problem the NAAB, the AF and NIADDK have set up a joint committee whose mandate is to critically review data available from national surveys as well as from other data sets of well-defined populations, and to publish the best available estimates on important parameters such as prevalance, incidence and economic costs. The group will keep in mind the problem of definition - both the clinical definition of the various forms of arthritis as well as the tendency of the clinician, the epidemiologist, and the lay community to define and report arthritis differently.

Rheumatoid Arthritis and Oral Contraceptives

Findings from three studies are suggestive of a negative association between the use of oral contraceptives (OC) and the development of rheumatoid arthritis. In reports from England and Holland, the incidence rate of rheumatoid arthritis (RA) among OC users was half the non-user rate. In a study of incidence and prevalence in Rochester, Minnesota, Mayo Clinic investigators noted that during the period 1950 through 1974, incidence rates for males remained relatively stable whereas rates for females declined dramatically during the last 10 years of the study. No explanation was found for the observed decline, but the authors believe that a factor introduced in the 1960s and acting selectively on females affected the incidence rates. Proposals were solicited for a case/control study of the risk of RA in relation to OC use and the effects of contraceptive steroids in Lupus: Studies in NZB/NZW FI Mice by the NICHD with NIADDK in an advisory role.

Raynaud's Phenomenon

Little is presently known concerning the prevalence of Raynaud's phenomenon.

The role of Raynaud's phenomenon as risk factor for scleroderma and other connective tissue diseases is poorly understood. Approximately 95% of patients with scleroderma suffer from Raynaud's phenomenon. Currently there are no reliable data to predict what proportion of patients with Raynaud's phenomenon will eventually develop scleroderma. Investigators at the University of South Carolina are attempting to identify criteria which will be useful in differentiating between Raynaud's phenomenon with benign or serious prognosis. Such information would be useful in the identification of a high risk Raynaud's phenomenon group and would alleviate the anxieties of subjects with benign Raynaud's phenomenon, if such proves to be the case.

Epidemiologic Activities in the Multipurpose Arthritis Centers

Nine centers have statistical and epidemiologic core units designed to enhance efficiency and effectiveness of the center's activities. The Stanford Biostatistical, Computer, and Education Methodology Core Unit, for example, provides consultation and review of all projects. It offers to center personnel expertise in biostatistics, computational consultation, intervention methodology, outcome measurement, and independent assessment.

The Research Evaluation and Support Core Unit (RESCU) at Boston University focuses on development and application of the Arthritis Impact Measurement Scales instrument. RESCU also works to coordinate and organize analyses of research data with the MAC. Data on amyloidosis, an area of special research emphasis in this center, are being computerized within this core.

At Brigham and Women's Hospital MAC, the Core Unit for Clinical Epidemiology and Evaluation in the Rheumatic Diseases, assists in formalization of research questions, aids in design of studies, and provides computerized methods for data storage and retrieval; and assists in statistical analyses.

The Centers also help development of individuals skilled in pursuing epidemiologic studies of rheumatic diseases. At the Johns Hopkins Center, a post-doctoral fellowship is available for a three-year program leading to a Masters of Public Health degree in epidemiology and full training in rheumatology. Epidemiologic training programs are also offered at the Rosalind Russell MAC (San Francisco) and at the Brigham and Women's Hospital MAC (Boston). In addition, epidemiologists familiar with other chronic diseases are being recruited. These programs may help to nurture the interest of young investigators in careers of rheumatic diseases epidemiology.

ARTHRITIS DATA SYSTEMS PROGRAM

The Arthritis Data Systems Program fosters systematic acquisition, storage, retrieval, and analysis of information concerning the rheumatic diseases. Program effort is focused on assuring validity, confidentiality and comparability of data collected in separate institutions, and integrating data resources with data needs. Rheumatic diseases data collection efforts and instrument development are prominent in three major settings: (1) American Rheumatism Medical Information System (ARAMIS) consortium administered from Stanford University, (2) Health and Nutrition Examination Survey (HANES I) Epidemiologic Follow-Up coordinated by the National Center for Health Statistics, in collaboration with NIH, and (3) Multipurpose Arthritis Centers.

A National Arthritis Data Resource (ARAMIS)

ARAMIS is a multi-institutional rheumatic diseases data bank system that currently contains 19,217 patients with 107,487 patient visits, representing 90,000 patient years of experience. The overall size of the data banks has increased by approximately 15% over the past year and two new data banks are on line. Data are prospectively gathered and efforts to maintain long-term patient followup are vigorous.

Goals of ARAMIS include: (1) improved diagnostic classification of rheumatic diseases, including establishment of acceptable criteria; (2) identification of "risk" factors associated with good or poor outcomes, based on prognostic studies among defined patient subsets; and (3) improvement of evaluation instruments for these purposes.

ARAMIS investigators and computer resources have continued to play an active role in studies of rheumatic diseases classification and prognosis. Within the past year, both the revised SLC Criteria and Juvenile Rheumatoid Arthritis Criteria were completed. Evaluation of the Rheumatoid Arthritis Criteria was also completed. Other classification studies in psoriatic arthritis, seronegative rheumatoid arthritis, vasculitis, myositis, and osteoarthritis are in progress.

In one followup study by ARAMIS Investigators, the Health Assessment Questionnaire (HAQ) was used to identify the predictors of future disability in moderate or severe rheumatoid arthritis (R.A.) patients. Three factors--radiographic grade, presence of symmetrical arthritis and age of the individual were found to be the most useful predictors of future disability of nearly 40 factors analyzed. Studies of long-term impact of disease on patients with R.A. are underway with regard to mortality, disability and cost. Preliminary findings indicate markedly increased mortality in R.A. patients and relate the deaths increase to initial disease severity. Recent studies include the effect of methotrexate in R.A., and the effect of anti-hypertensive drugs in treatment of scleroderma. An instrument to measure side effects has been developed and is currently being validated. Another instrument, to measure both direct and indirect economic impact, has been completed and added to the ARAMIS outcome assessment protocols.

Health and Nutrition Examination Survey (HANES) Epidemiologic Followup Survey

In cooperation with the National Institute on Aging (NIA), the National Center for Health Statistics (NCHS) and several other Institutes, the Arthritis Epidemiology and Data Systems Program participated in designing the instrument for collection of data in this resurvey of 14,407 individuals, originally sampled in 1971-1975.

The followup instrument will serve four principal study objectives: retrospective morbidity and mortality associated with suspected risk factors; prospective mortality associated with suspected risk factors; changes in patient characteristics between HANES I and current status; and natural history of chronic disease and functional impairments.

The HANES Followup Survey is completed and data collection for the extensive arthritis section has been free from serious difficulties. Plans are underway by the participating institutions to coordinate and cooperate in data analysis. In addition, plans have begun to follow the cohort for an additional time period.

The Multipurpose Arthritis Centers (MACs)

MACs continue to be active in the development, refinement and application of health status measures (HSM). For example, recent studies carried out by Boston University Arthritis Center investigators examined the ability of one HSM to detect small improvements in health. Standard measures were compared to the Arthritis Impact Measurement Scales (AIMS) instrument in a drug trial. Both measures gave essentially identical results. Thus the AIMS instrument was reported to be quite sensitive to clinically meaningful, drug induced improvements and may be useful in clinical trials in rheumatology.

ANNUAL REPORT

DIVISION OF DIABETES, ENDOCRINOLOGY, AND METABOLIC DISEASES

OFFICE OF THE DIVISION DIRECTOR

This office plans and directs programs of research grants and contracts, training and fellowship awards, and special programs and activities within the Diabetes, Endocrinology, and Metabolic Diseases Division. The Division Director is responsible for strengthening, integrating, and coordinating activities within these areas and for developing and initiating new programs to meet the needs of the scientific community.

In the area of diabetes, the Division Director, acting through the NIH Diabetes Coordinating Committee, has primary responsibility for coordination of all trans-NIH activities relating to diabetes mellitus and its complications. This provides a centralized focus for the planning, development, implementation, and assessment of program initiatives among each of the NIH Institutes that support diabetes-related research. The Division Director also serves as Chairman of the Diabetes Mellitus Interagency Coordinating Committee, whose membership includes representatives from all Federal agencies which conduct or support activities related to diabetes mellitus. He also serves as an ex officio member of the National Diabetes Advisory Board, as Co-Chairman of the NIH Cystic Fibrosis Coordinating Committee, and he plays a lead role in liaison with voluntary health groups and scientific societies concerned with diabetes, endocrinology, and metabolic diseases.

Although not detailed below, the Division Director and his staff also serve as an information resource to the scientific community, lay public, and other Federal, state, and private organizations with respect to the full spectrum of research, research training, and information-transfer activities in diabetes, endocrinology, and metabolism. In addition, they contribute to a wide variety of NIADDK and NIH-sponsored activities to further the continued development of expanded scientific capabilities in other related areas of biomedical research.

DIABETES PROGRAMS BRANCH

This Branch administers the Division's Diabetes Research, Diabetes Centers and Clinical Trials Programs. In addition, the office of the Branch Chief provides an administrative focus for planning and implementing special initiatives and for coordinating support of diabetes-related awards in other NIADDK programs.

Diabetes Research Program

During the past year, the Diabetes Research Program supported research grants and contracts in a variety of scientific disciplines which continue to contribute to the fundamental knowledge base needed to define the disease and its complications. Work continues on the development of improved medical capabilities for prevention, diagnosis, treatment and cure of this condition. These studies focus primarily on defining the natural history, etiology, epidemiology and pathogenesis of diabetes and the resulting complications. Areas of particular research interest include studies involving islet cell transplantation; insulin delivery secretion, transport, action, interaction, and inactivation of islet hormones; the physiology, pathophysiology of pancreatic islet cells; abnormalit of carbohydrate, lipid, and protein metabolism associated with diabetes and its complications; the possible relationship between various genetic, viral, immunologic, toxicologic, and nutritional parameters in the etiology and pathogenesis of diabetes; and the psychosocial aspects of diabetes. Examples of progress in some of these areas are summarized below:

Passive Transfer of Diabetes in the BB Wistar Rat

The BB rat, a spontaneous diabetic animal model, has become an important adjunct to this area of research. It appears that this animal most closely resembles human Type I Diabetes (IDDM). It has been important in the development of the concept that one type of diabetes appears to be a slow progressive process that only becomes apparent when the loss of a sufficient number beta cells (about 80%) leads to hyperglycemia.

The NIADDK awarded a contract (Jan. 1984) to the University of Massachusetts to produce a limited number of these animals for distribution to the research community in order to stimulate new and important research.

A recent study using the diabetic BB Wistar rat demonstrated the passive transfer of the disease to a "control" line of non-diabetic rats. This was accomplished by using Concanavalin A - treated (Con-A) spleen cells from animals with acute diabetes. As we reported in a previous year, administration of anti-lymphocyte antiserum prior to or at the time of onset of hyperglycemia is useful in preventing the development of diabetes. These studies together with neonatal thymectomy and bone marrow transplantation studies in diabetic animals suggest a role for cell-mediated immunity in the pathogenesis of the disease.

There has been evidence that insulitis can be produced in a non-diabetic animal by the passive transfer of cells from affected animals. The present study, using Con-A treated donor spleen and lymph node cells from diabetic rats showed passive transfer of hyperglycemia to some controls and to diabetes-prone animals at an earlier time than usually occurs. Two thirds of the controls treated with the Con-A treated donor spleen and lymph node cells were still euglycemic at 118 days while one third showed hyperglycemia at 86 days. The earliest appearance of diabetes in the diabetes-prone animals is usually 60 days. Further experiments are needed to determine the precise role of Con-A in activating the cells so that they transfer insulitis and diabetes and to identify the specific cell type(s) responsible for the transfer. This model may help to delineate the beta cell antigen against which the presumptive pancreatic autoreaction is directed in the spontaneously diabetic BB Wistar rat.

The BB Wistar rat has made possible the study of the role of autoimmunity in the pathogenesis of IDDM. The passive transfer of diabetes and insulitis provides strong support for a role for cell-mediated immunity in the pathogenesis of the disease.

Further studies are needed to determine the precise role of Con-A in activating cells so that they transfer insulitis and diabetes and to identify the specific cell type(s) responsible for this transfer.

Evidence for at Least Two Distinct Forms of IDDM, Each Associated with Specific HLA Alleles

Increasing evidence supports the notion that certain HLA antigens, B8, B15, DR3 and DR4 specifically, are present at increased frequences in diabetic individuals relative to the population as a whole. While it was observed that some forms of IDDM were associated with the development of autoimmunity and other forms were not, the relative association of these symptoms to specific HLA alleles is not understood.

On the basis of accumulating evidence, one group of investigators has proposed a hypothesis to explain this heterogeneity within IDDM. The hypothesis states that there are at least two distinct forms of IDDM, one associated with HLA B8 and DR3 and another with HLA B15 and DR4. The HLA-B8/DR3 disease is the autoimmune form associated with an increased presence of pancreatic islet-cell antibodies and cell mediated immunity directed against the pancreas and a lowered antibody response against externally supplied insulin. This form of diabetes has a probable onset throughout life and may be responsible for those cases of IDDM where onset occurs after the age of twenty. The second form of diabetes is associated with the HLA-B15/DR4 antigens. This disease is not associated with autoimmune disease or islet cell antibodies, but is accompanied with an increased antibody response to insulin, possibly after viral induced damage to insulin secreting cells and a more rapid onset of the disease.

These proposed types of diabetes also explain the course of diabetes in those individuals unfortunate enough to be compound heterozygotes, i.e.; who have both B8/DR3 and B15/DR4 alleles. This group appears to have the earliest age of onset of IDDM, the greatest islet cell damage, the lowest levels of measurable C-peptide and the highest rate of concordance among identical twins. The model that the investigators propose to explain this phenomenon is that the DR3 and DR4 antigens act through different pathogenetic mechanisms, and that in an individual with both alleles, the diabetogenic effect is synergistic, leading to increased susceptibility.

Identification of the various antigens associated with diabetes may lead to the elucidation of the gene or genes that actually allow the initial insult to take place. The proposed model provides an explanation for how the observed multitude of symptoms and characteristics reflects the inherited genetic differences in individuals. This model may allow researchers to accurately assess the role of environmental factors as opposed to the role of inherited genes. It may also help to formulate more accurate estimates of the risk for diabetes and more effective treatment strategies.

Identification of HLA-D Fragments that Differ Between Diabetic and Non- Diabetic Individuals

With the advent of the various recombinant DNA techniques, many investigators have identified and isolated previously inaccessible unique gene sequences within the mammalian genome. Investigators working with the human major histocompatibility complex (MHC) have elucidated the structures of some of the HLA antigens. The HLA region genes are expressed as antigens called either Class I or Class II antigens. Class I antigens include HLA-A B and C types and are composed of two polypeptide chains. The HLA-D/DR antigens comprise a group of antigens called class II and are also composed of two polypeptide chains, termed alpha and beta. Recently, investigators have shown that there is only one gene which codes for the alpha chain but there are at least three genetic loci that code for the beta chains. Now, using a clonal probe of the region encoding the beta chain, researchers have looked at the DNA patterns from healthy individuals and individuals with diabetes after digestion with various restriction endonucleases. Individuals were chosen who had either HLA-DR3, HLA-DR4 or both in their genomes. Samples of the DNA were each cut with three separate enzymes, Eco RI, Bam HI and PstI. There was no difference in the DNA fragments cut with Eco RI between the healthy and IDDM individuals. However, there were quite a few differences in the Bam HI, and PstI cut DNAs from these two groups. Among the HLA-DR4 and HLA-DR3/4 individuals, the IDDM patients showed an increased frequency of a PstI 18 kilobase (kb) fragment. In addition a Bam HI 3.7 kb fragment, frequent among controls (20-40%), was rarely detected in IDDM patients. It is thought that these differences are associated with the DR4 alleles, rather than the DR3 allele.

These analyses point to the possibility that there is more than one type of DR4 allele, one more strongly associated with IDDM than another. If the DR4 allele in IDDM individuals confers a special susceptibility to diabetes, this could account for the increased risk of disease that is observed in sibs of the affected individuals. Therefore, the DR4 allele may be considered to be a family of antigens which react in a similar fashion serologically, but are in fact different genetically. Identification of the "diabetogenic" allele could answer questions on the mode of inheritance of IDDM, and the role that this antigen plays in the susceptibility to the disease.

Population Studies of the Increased Prevalence and Incidence of Diabetes in t South Pacific Islands

Two recent studies examined the role of diet in two different populations in the South Pacific. Dietary studies were conducted in Kiribati and in Nauru in conjunction with a diabetes and cardiovascular disease prevalence survey. Both of these populations have recently undergone urbanization.

In Kiribati, twenty-four hour dietary recall data were obtained from 1062 randomly selected adults, 20 years and older. These samples were from both rural and urban groups. The percentage contributions of protein, fat and carbohydrate to the total energy intake did not differ between rural and urban Kiribati but the urban group consumed an increased amount of imported foods which contributed to their total energy intake.

Urban dwellers consumed more rice, sugar, drippings, canned meats and fish, condensed milk and bread and less coconut, coconut sap, taro and pandanus than their rural counterparts. Energy intake of rural males was significently higher than those of urban males. There was no statistical difference between energy intake of urban and rural females.

Age-standardized diabetes prevalence was 9.1% and 8.7% for urban males and females respectively and 3.0% and 3.3% for rural males and females. It was apparent that the urbanization in Kiribati is accompanied by a marked change in food habits, a decreased level of physical activity, obesity, and an increased prevalence of non-insulin dependent diabetes.

In a similar study in Nauru it was determined that although the contributions of protein, fat and carbohydrates were similar for the Nauruans and the Caucasian population on the island, the average dietary fiber intake of the Nauruans is much lower than that of the Caucasians. Thiamine intakes were found to be below recommended dietary allowances and the ascorbic acid intake was found to be marginal in susceptible groups.

Diabetes prevalence rates for adult male and female Nauruans are amongst the highest reported, 24.7% and 23.5% respectively. Marked obesity is evident also in these people.

It appears from these and other studies on these groups that in the Nauruans gross obesity and a high degree of urbanization contribute to the disease whereas in the Kiribati both adiposity per se and urbanization independent of obesity are associated with the increased risk for developing diabetes. This type of information is contributing substantially to our knowledge of not only the genetic heterogeneity associated with the disease but also the diverse environmental factors that are involved.

Studies of such isolated populations, at risk for developing diabetes, can provide a great deal of insight into the cause of this disease in Western populations. It is possible in these isolated populations to assess the relative contribution of heredity, diet and exercise to the development of diabetes and its complications.

Increased Risk of Hypoglycemia Due to Impairment of Counterregulatory Hormones During Intensive Insulin Therapy

Intensive insulin therapy such as the use of insulin pumps has been successful in reducing hyperglycemia and maintaining normal levels of glycosylated hemoglobin (as indicator of glucose control). However, this therapy can also lead to severe hypoglycemia in some patients. In Type I diabetics, the glucose response is commonly blunted or absent and these individuals become dependent upon epinephrine to promote glucose recovery from hypoglycemia. Recent evidence indicates that epinephrine secretion is diminished as a result of diabetic autonomic neuropathy.

Patients who have diminished epinephrine secretion as well as impaired glucagon response are at risk for recurrent severe hypoglycemia during intensive insulin therapy and their early identification should be attempted. A test was recently developed by grantees of NIADDK to detect these individuals. Twenty-two diabetics and 10 controls were stabilized at 80-120 mg/deciliter plasma glucose (overnight) and then infused with insulin for 100 minutes in the morning. Plasma glucose levels were determined frequently and patients were monitored for any signs of the neurologic manifestations of hypoglycemia. The controls produced an increased level of insulin, decrease in plasma glucose level, increase in glucagon, epinephrine, norepinephrine and cortisol and no signs of hypoglycemia or neurologic symptoms. Thirteen of the 22 diabetics showed adequate counterregulation which was not a result of glucagon increase but of an epinephrine response. The epinephrine response was markedly reduced in the nine patients with inadequate counterregulation. After completion of this test all 22 patients were treated with intensive insulin therapy for 6-26 months. During that period only one patient who had been classified as having adequate control had two episodes of severe hypoglycemia while 46 episodes of severe hypoglycemia occurred in eight of the nine patients classified as having inadequate control. All episodes required administration of intramuscular glucagon or intravenous glucose.

This insulin infusion test appears to meet the objective of identifying IDDM patients at increased risk for recurrent severe hypoglycemia during intensive insulin therapy.

Advances in the Characterization of the Structure of the Insulin Receptor and its Insulin-Mediated Phosphorylation

Important new studies have occurred in the area of the insulin receptor and its characterization. Several investigators have documented that there are two major polypeptides constituting the insulin receptor. These subunits have molecular weights of approximately 135,000 (alpha) and 95,000 (beta). A larger polypeptide (210,000 on SDS gel electrophoresis) is seen in small quantities and probably represents a precursor of one or both of the other subunits. The alpha and beta subunits appear to be disulfide-linked in the native receptor and to exist as one or more types of complexes. The alpha subunit appears to be the insulin binding subunit and the beta subunit appears to be the effector subunit.

Further work has shown that both the alpha and beta subunits are phosphorylated by the action of insulin. The beta subunit phosphorylation is five times that of the alpha subunit. The phosphorylation of the beta subunit of the receptor is stimulated 2.5 - 3.5 fold by insulin. In whole cells, this stimulation is due mainly to an increase in the content of phosphoserine and the appearance o phosphotyrosine. When isolated receptors are studied, the overwhelming majori of insulin-stimulated phosphorylation is on tyrosine residues of the beta subunit. Studies in several cell systems suggest that the phosphorylation appears to be catalyzed by protein kinase activity in the receptor itself and the hypothesis has been proposed that the beta subunit of the receptor is a tyrosine protein kinase.

These findings suggest a complex model for insulin receptor synthesis and its location and role in the membrane.

The rapidity and specificity of the action of insulin on its receptor suggests that insulin-mediated phosphorylation is one of the earliest steps in insulin action following binding of the hormone to its receptor.

Phosphorylation/dephosphorylation which has been shown to be an important step in the action of many protein messenger molecules, is proving to be the case with the insulin as well.

Effects of a Long-Acting Somatostatin Analogue on Postprandial Hyperglycemia in Insulin-dependent Diabetes Mellitus

Studies have demonstrated that the administration of des-Alal,Gly2(His4,5, D-Trp8)-somatostatin analogue (WY-41,747) significantly enhanced the effectiveness of subcutaneously administered insulin in limiting the postprandial hyperglycemia of insulin-dependent diabetes. Moreover, administration of the analogue along with insulin permitted satisfactory control of postprandial hyperglycemia when the subcutaneous insulin infusion was initiated immediately before meal ingestion. Such an agent may prove useful as an adjunct to insulin in the management of diabetes mellitus.

Identification of the Process Through Which Newly-Synthesized Insulin is Stored or Secreted

In a recent study, investigators incubated islet cells, in vitro, with radioactive leucine for a brief period in order to label newly synthesized proteins. After removal of the radioactive protein precursor, islets were exposed to buffer containing either high or low concentrations of glucose in a specifically timed sequence which corresponded to that interval in which secretory vesicles are formed and proinsulin is converted to the fully mature insulin protein in the Golgi apparatus of the cell. The investigators then challenged the pre-labeled islets with combinations of glucose and other physiologic or therapeutic compounds to determine what would cause a preferential release of the newly synthesized, radiolabeled insulin. Usually, insulin is stored in these vesicles until the islet cell releases this hormone as part of normal secretory events, or in response to an external secretory challenge. The results in these experiments show that islets previously exposed to a low concentration of glucose release both old and new insulin in response to a glucose or therapeutic challenge. However, islets initially exposed to high concentrations of glucose, preferentially release newly synthesized insulin in response to either a glucose challenge or glucose in combination with other chemical or therapeutic substances.

The investigators concluded that exposure to high concentrations of glucose during the critical time of transfer of insulin to secretory vesicles "marks" those vesicles for immediate release of hormone at a subsequent glucose challenge. This "marking" process allows the cells to secrete insulin in a non-random way and is perhaps, regulated by a novel, glucose activated secretory route. In contrast to the normal islet cells, secretory vesicles of an islet cell tumors did not respond to "marking" by glucose, nor do they show differences in secretion of old and new insulin when stimulated by various secretagogues, thus suggesting that these tumor cells had aberrant storage and secretion characteristics.

Use of Ultraviolet-Treated Blood from a Potential Donor to Overcome Transplantation Rejection in Allograft Tissue

A recent study demonstrated a rapid and simple method for generating Ia depleted donor blood for preimmunization by treatment with ultraviolet (UV) light. For many years, it has been known that treatment of blood cells with UV light eliminated the capacity of these cells to stimulate other lymphocytes to divide in the classic mixed lymphocyte reaction (MLR), a standard immunological tool used to test compatibility of donors and recipients. The investigators hypothesized that for the purposes of preimmunization, the Ia bearing cells need not be eliminated from the blood, but only inactivated, leaving the rest of the immune system intact. The blood was diluted in a sterile saline solution, placed in petri dishes and exposed for 20 minutes to UV lights positioned 10 centimeters away from the surface. Recipient diabetic rats were injected with UV treated blood from donor rats prior to receiving islet cells from rat donors.

Diabetic animals who were thus treated, showed 100 percent conversion to normoglycemia for a period of over 160 days after allografting.

Other animals who were either not immunized or immunized with blood from a third unrelated species before transplantation, showed mean survival time of the grafts of only 8 days posttransplantation. In addition, those animals which were immunized with nonirradiated blood from rat donors also rejected the islet cell by the 7th or 8th day after transplantation.

Related in vitro studies, using monoclonal antibodies against Ia antigens, conclusively determined that although there was no difference in the binding of the Ia antibody to the Ia antigen on the blood cells in irradiated or untreated blood, the irradiated blood cells failed to cause stimulation in the MLR. These results demonstrate that an activity of the Ia bearing cell is required to initiate the rejection process and that transfusion of UV-irradiated whole blood from donors can lead to prolonged survival of islet cell grafts in recipents. This treatment appears to induce donor specific unresponsiveness and this result can be obtained without the use of immunosuppressive drugs. The induction of the rejection process apparently requires cells that are metabolically active; UV irradiation inactivates the causative cells so that the host becomes unresponsive. This method could be applied to other species where specific antisera are not available.

If prolonged survival of human allografts can be achieved by this procedure, it may be possible to eliminate the use of immunosuppressive agents to maintain transplanted tissue.

Use of Sorbinil for Relief of Pain from Diabetic Neuropathy and Improvement of Somatic and Autonomic Nerve Function

Aldose reductase is the rate-limiting enzyme in the conversion of hexose sugars to sorbitol. Intracellular accumulation of sorbitol occurs when intracellular levels of glucose are high. A recent study of the potent aldose reductase inhibitor, sorbinil, was performed on 11 subjects (6 males, 5 females, all Caucasian - 7 Type I and 4 Type II diabetics) with mean duration of diabetes for 16 years. All individuals required insulin, and all patients were incapacitated by severe pain attributable to diabetic neuropathy. Eight of the eleven alternately received the drug and acted as their own control while the other three only received the drug having received other treatment without success. In summary, eight of the original eleven had moderate to marked relief of symptoms from the drug, two had equivocal responses and one had no change. Results varied in the various patients but there was evidence of improved muscle strength and sensation, autonomic nerve function and autonomic nerve conduction velocities. This investigation suggests the potential for this class of agents as a treatment for symptoms of diabetic neuropathy. Although improvement was not universal, clinical improvement seemed to be related to increase in nerve conduction velocity. These observations must be interpreted with caution since a number of authors have drawn attention to the lack of correlation between nerve conduction measurements and symptomatic improvements. On the other hand, other studies have documented good correlations between nerve conduction velocities and nerve function. The authors suggest that function might well be demonstrable with prolonged drug administration. Although these studies suggest a possible metabolic etiology related to polyol or sugar alcohol accumulation the exact mechanism needs to be defined since improvement was seen in patients who had good glucose control at a level below which the sorbitol pathway is believed to be operative.

These and other studies support the role of sorbinil as a primary agent in treating the effects of diabetic neuropathy. This drug could relieve some of the morbidity associated with the neurologic complications of diabetes.

Clinical Trials Program

The Diabetes Control and Complications Trial (DCCT) is a multi-centered (21), controlled clinical trial which has been designed to determine if maintenance of near-normal levels of blood glucose in patients with insulin-dependent diabetes mellitus (IDDM) can prevent, delay, or ameliorate the microvascular and neurologic complications of IDDM. The study was designed to have four phases: planning, feasibility, full-scale clinical trial, and data analysis and reporting.

Phase I of the trial was completed in FY 1983; Phase II was initiated in August 1983 with commencement of the recruitment of volunteers. The DCCT completed recruitment and randomization of 278 volunteers for its feasibility phase (Phase II) in March 1984. Feasibility of moving on to the full-scale clinical trial (Phase III) will be determined on the basis of analysis and review of one year's of follow-up data on each subject. Key feasibility questions which need to be answered are whether a statistically significant and clinically meaningful differential in blood glucose control can be achieved and maintained; whether the biochemical and clinical outcomes of the study can be measured and documented with acceptable precision; and whether the treatment regimens are safe and acceptable to subjects. Recruitment goals were met essentially on time and all volunteers accepted their treatment group assignments.

Financial support for designated components of the DCCT has been forthcoming from the NINCDS and the NHLBI. Following *ad hoc* peer review and consideration by their Council, the NINCDS agreed to provide full support for inclusion of a neurological component in FY 83 and FY 84. These funds are being provided via intra-agency agreement and have been applied to the cooperative agreement research grants and the research contract. The NHLBI has agreed to support the cardiovascular component in FY 83 and FY 84 and to provide surplus ECG machines for use in the DCCT clinics. NHLBI funds were provided through direct transfer to the research contract to support central reading of ECGs, lipid analyses by the central biochemistry laboratory, and data management costs related to this aspect of the protocol. In addition, the Division of Research Resources has actively promoted the DCCT among the directors of the General Clinical Research Centers (GCRC). Seventeen of the 21 participating DCCT centers have GCRCs and the DRR has encouraged the directors to facilitate use of these centers by the DCCT investigators. The National Eye Institute has contributed study materials as well as invaluable technical assistance in development of study documents. In addition, various commercial organizations have donated supplies, equipment, and services.

Diabetes Centers Program

This program provides support for both Diabetes-Endocrinology Research Centers (DERC) and Diabetes Research and Training Centers (DRTC). The primary requirement for establishment of either a DERC or a DRTC is a strong base of high-quality, ongoing biomedical research. DRTCs have, in addition to a research component, a training/information transfer component which includes training of both medical and allied health professionals, continuing education, model demonstration, and outreach activities. For both types of centers, the grant provides for core facilities (shared resources), pilot and feasibility studies (new initiatives), and limited funds for program enrichment. This funding is intended to strengthen existing programs, increase productivity, and foster the generation of new ideas and innovative research approaches. Although each center is expected to develop its own independent program in accordance with local needs, interests and resources, each is also expected to be responsive to national needs, and must be willing to work cooperatively with the NIADDK and, in the case of DRTCs, with other Federally-supported diabetes programs.

Presently Funded Centers

During the past year, the program provided support for 5 DERCs and 7 DRTCs; Diabetes Endocrinology Research Centers - University of Pennsylvania (Philadelphia), University of Washington (Seattle), University of Iowa (Iowa City), Baylor School of Medicine (Houston), and University of Massachusetts (Worcester): Diabetes Research and Training Centers - Albert Einstein College of Medicine (Bronx), Indiana University (Indianapolis), University of Michigan (Ann Arbor), Washington University* (St. Louis), University of Chicago* (Chicago), Vanderbilt University* (Nashville), and University of Virginia* (Charlottesville). All but two of the present twelve centers have undergone at least one competitive renewal review. Since establishment of the program only one center has not received continued funding. Two renewal applications from centers have undergone review during FY 1984. The DERCs and DRTCs are being evaluated continually through several varied but complementary processes which include NIH peer review, review of progress reports, staff visits to centers, annual meetings, special evaluation projects by Institute staff, and in house evaluations by centers themselves.

A mechanism for support has also been developed which allows DERCs the opportunity to become DRTCs. A DERC may apply for a modest exploratory grant to develop the resources necessary for initiation of DRTC training and information transfer activities.

Description of the Centers

While the DERCs have only a Biomedical Component, the DRTCs have two major components - the Biomedical Component and the Training and Information Transfer Component. Within each component there are three types of activities for which center funding may be proposed: (1) shared resources (cores); (2) pilot and feasibility studies; and (3) enrichment. Each of these activities will be briefly described.

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*Previously supported as a DERC

A biomedical core at a center is a shared facility which provides a service needed by and available to center research investigators to more efficiently conduct biomedical research. In addition to the provision of a specific service (e.g. radioimmune assays or molecular biology methodologies) the cores serve as a focal point for collaboration; provide consultation and training in highly sophisticated technical areas; provide ready availability to large expensive equipment and more efficient use of this and other equipment; pursue limited developmental work to maintain the latest and best methodologies; enhance clinical research; and generally provide service at a cost benefit to investigators.

The training and information transfer cores of the DRTCs also provide services, but of a different character, for example: biostatistical consultation, design of evaluation instruments, developmental design of programs and materials, and (in the case of the Model Demonstration Unit) short term training of health professionals and enhanced opportunity for clinical research.

Pilot and feasibility studies provide modest research support in diabetes-related areas for a limited time period (three years of less) for exploring the feasibility of a concept and amassing sufficient data to allow the investigator to apply for independent funding through regular research grant mechanisms.

Enrichment constitutes enhancing the multidisciplinary environment through seminar and conferences, and consultation and lectures from outside the center institution.

All of the funding in the DERCs is for biomedical research. In the DRTCs, the proportionate funding for the three fundable activities varies from center to center, since institutions are encouraged to build on their strengths. However, an average of 62% of DRTC funding is devoted to biomedical research, including an average of 46% for biomedical core resources and 16% for pilot and feasibility research projects. For the training and information transfer component, which also embraces some clinical research support, an average of 38% of the funding is used. Less than 1% of funding in any of the centers is used for enrichment activities.

A primary and most important prerequisite for the establishment of either type of center is an already existing substantial base of high quality, independently supported research in diabetes and related endocrine and metabolic disorders. The center provides (1) services from shared resources (cores) for the center key participants; (2) pilot and feasibility research studies for young investigators and investigators new to the field of diabetes; (3) activities in training and information transfer in the DRTCs; and (4) enrichment of the total research and training efforts. This is intended to create an environment in which all aspects of the center - research, research training, and (in DRTCs) training of health professionals - are substantially enhanced.

Another goal of the centers is the development of a multidisciplinary approach by bringing together into the center environment investigators from other disciplines relevant to diabetes research. Several unique features of the centers contribute significantly to this effort; (1) sharing by new investigators and investigators from other disciplines in the services of the shared resources or cores; (2) availability of pilot and feasibility studies to attract new investigators; (3) consultation about and/or training in highly technical procedures offered by the cores; and (4) participation in the multidisciplinary environment and enrichment opportunities offered by the center. Selected research highlights from the centers are included in the report of the Diabetes Research Program.

The Training and Information Transfer Component of the DRTCs was a new and innovative approach in the diabetes field at the time of their initiation. Heretofore, opportunities for training in this area were few, and most often purely didactic with no meaningful evaluation or follow-up on outcomes. The DRTCs were mandated by the legislation to do more than this. Now, 6-8 years after their establishment, a host of programs and materials have been developed which reflect the most advanced and substantiated approaches for health care professionals in diabetes education, care and treatment. A significant number of these have been tested and evaluated and are generally available to the diabetes community. These materials are deposited with the National Diabetes Information Clearinghouse (NDIC) and are described in a compendium, along with other similar programs and materials, which has been compiled by the NDIC. Use of these materials outside centers understandably varies, but overall the contribution of the centers to the availability of programs and materials for training of health professionals is keeping pace and complementing efforts from other sectors to improve on a widespread basis through training of health care professionals the education and treatment of diabetic patients.

One of the other mandates of the DRTCs was to develop liaison and cooperative efforts with other Federal agencies and organizations in the private sector with interests in diabetes. Previous reports have dealt with the development of collaborative arrangements and consultations, particularly with the CDC Diabetes Demonstration Projects and the Model Care Diabetes Program of the IHS. Materials and Programs developed by the centers have been used or adapted for use in other settings, and DRTC personnel have acted in a great many instances in a consultative capacity, primarily for the CDC State projects, the IHS, and somewhat more recently, the DCCT.

SPECIAL PROGRAMS BRANCH

This Branch administers the National Diabetes Data Group and National Diabetes Information Clearinghouse, and the Manpower Development Program.

National Diabetes Information Clearinghouse

The major function of the National Diabetes Information Clearinghouse (NDIC) continues as the central point for the collection and dissemination of information about materials, programs, and resources relevant to diabetes. Over 4,000 pieces of diabetes educational materials that are available for purchase or at no cost have been identified and abstracted. Because health care providers often need information about a variety of health conditions, the director of the NDIC has taken the lead in developing a Combined Health Information Data Base. The Centers for Disease Control, the Office of the Assistant Secretary for Health, DHHS, and other information dissemination programs at the NIH have enthusiastically joined forces with the NDIC. After the new data base has been thoroughly tested, every effort will be made to interest one or more major vendors in assuming all costs for its maintenance. Answering requests for information is a continuing NDIC activity. The NDIC receives over 1,500 letters and phone calls a month asking for information about every aspect of diabetes and self-care practices.

The Diabetes Dictionary, developed by the NDIC in cooperation with several voluntary and professional diabetes organizations, was published by Eli Lilly and Company as a public service. To date, the NDIC has distributed 20,000 copies. Eli Lilly and Company is now printing another 50,000 copies and will assist in the distribution of this publication.

National Diabetes Data Group

The Data Group serves to foster, coordinate, and integrate national resources for the collection, analysis, and dissemination of data about diabetes in order to provide a sound basis for the development of scientific priorities and the planning of public health programs. The Data Group also continues to provide the Federal focus to which the lay and scientific communities turn for accurate, reliable statistics on diabetes.

The National Diabetes Data Group consists of a nationwide group of expert advisors including epidemiologists, individuals with expertise in the biomedical, nutritional, and socioeconomic aspects of diabetes, and representatives of Federal agencies (the National Institutes of Health, the Centers for Disease Control, the National Center for Health Statistics, and the National Diabetes Advisory Board) and the private sector (the American Diabetes Association and the Juvenile Diabetes Foundation).

The Data Group has embarked on three major projects:

(1) Development of a comprehensive data book about diabetes to respond to the data needs of researchers, public health officials, clinicians, and other persons and agencies concerned with diabetes. In this project, the Data Group continues to work in close collaboration with the National Center for Health Statistics (NCHS) in assessing the national data on diabetes collected by that agency. The NCHS Divisions of Analysis, Mortality Statistics, and Health Care Statistics are participating in development of the comprehensive data book by supplying data on multiple causes of death among diabetics, sociodemographic characteristics of diabetics, and medical care aspects, including ambulatory, hospital, and nursing home care. The Centers for Disease Control are collaborating by assessing hospitalizations for diabetes and acute metabolic complications. Many epidemiologists in U.S. universities and medical schools are also contributing to this data compilation and assessment.

(2) With the Division of Health Examination Statistics, the Data Group has analyzed oral glucose tolerance tests in a statistical sample of the U.S. population to develop norms and standards for plasma glucose levels and to determine the prevalence of undiagnosed diabetes and impaired glucose tolerance. This Division is also conducting a study of Hispanic-Americans, for which the Data Group developed the medical history and household questionnaire and the clinical and lab tests to be used. The Data Group is also collaborating with the Epidemiology and Field Studies Branch, NIADDK, in assessing NCHS survey data to examine the issues of screening for diabetes, etiologic relationships in undiagnosed diabetes and impaired glucose tolerance, obesity, nutritional factors, and family history of diabetes.

(3) The Data Group has taken the lead in establishing a consensus on classification and diagnostic criteria for diabetes. An Expert Committee has been convened to review and assess data collected since the 1979 Data Group report. This report will serve as the American recommendations to the WHO Study Group on Diabetes, and it is anticipated that the American consensus may influence an international consensus, such that future research data may become more comparable, facilitating our ability to compare diabetes incidence and complications in populations with differing genetic and environmental characteristics.

Manpower Development Program

The mission of the Division's Training Program is to provide research training for the most promising investigators in diabetes, endocrinology, and metabolic diseases. The number of applicants for National Research Service Awards remains high and approximately 350 positions (both individual and institutional) currently are being funded. The institutional allowance continues at the FY 82 decreased level for both individual fellowships and institutional training grants.

The Division supports three types of career development awards: The Physician Scientist Award (PSA), which provides a first experience for newly trained clinicians to develop independent research skills and experience in a fundamental science; the Research Career Development Award (RCDA), which provides salary support for 5 years to the investigator who has demonstrated research potential but who needs some additional research experience; and the Clinical Investigator Award (CIA) which is meant specifically for individuals with clinical training who need the opportunity to develop into independent biomedical investigators. During FY 84, 27 awards were made in these 3 programs.

ENDOCRINOLOGY AND METABOLISM PROGRAMS BRANCH

This Branch administers the Division's Endocrinology and Metabolic Diseases Research Programs. In addition, the Office of the Branch Chief provides an administrative focus for planning and implementing special initiatives and for coordinating activities related to cystic fibrosis.

Endocrinology Research Program

The program's major emphasis is on basic research into the mechanisms of hormone action, hormonal control mechanisms, and synthesis of hormones. A smaller but significant portion of the program effort is devoted to clinical research. Studies of thyroid, parathyroid, adrenal, pituitary, thymus, and pineal hormones are supported, as well as studies on the neuropeptides and prostaglandins. Examples of progress in some of these areas are summarized below.

The Structure of Pre-pro-enkephalin has Been Determined

By the use of cDNA probes, a messenger RNA sequence was identified that is considered to be the primary structure of human Met and Leu-enkephalin. It consists of 267 amino acids. The interesting feature of this protein sequence is that is consists of six molecules of Met-enkephalin and one molecule of Leu-enkaphalin. The enkephalins are scattered through the long molecule and, at present, there is no known activity for the remaining peptide segments.

In addition, each enkephalin molecule in the precursor molecule is flanked on both sides by pairs of basic amino acids (lys-lys or lys-arg). These amino acids appear to be the site of cleavage of trypsin-like enzymes, thus it would seem that the enkephalins are marked for enzymatic cleavage from the parent molecule.

Leu- and Met-enkephalin are peptide hormones which contain five amino acids each and are identical except for the terminal amino acid which is either Leucine or Methionine. They have opioid activity, that is, they are naturally occuring hormones which have a morphine-like effect on pain in the body.

Met-enkephalin is produced in the pituitary gland as part of a larger molecule known as pro-opiomelanocortin (POMC) which also produces several other hormones. The enkephalins are also produced in the adrenal medulla. The object of this research was to establish in what form enkephalin is produced in the adrenal.

Since the complete sequences of both pituitary and adrenal medulla sources of enkephalin precursors have now been established, and are shown to be different, it is now known that the enkephalins from each source are produced by different genes. In addition, it is of interest to note that several molecules of the same hormone are produced by each precursor, indicating an economy of effort in production.

Research of this type has been and will be applied to the production of hormones in bacteria for commercial use. In addition, this type of research is useful in determining how hormones and other products are produced in the body, in order to improve our overall knowledge of body mechanisms.

Growth Hormone Releasing Factor (GRF) Effects on Growth Hormone (GH) and Somatomedin C Levels in Humans

Twelve adult patients who had been diagnosed and treated in childhood for growth hormone deficiency were injected with synthetic GRF-40 (10 micrograms per kilogram of body weight) on two consecutive days. Blood samples were drawn at 15 or 30 minute intervals for 3 1/2 hours and GH and other pituitary hormones were measured in the blood samples. GH secretion was stimulated in some but not all of the patients, but to a lesser extent than in normal controls. These results are interpreted to mean that some GH deficiency is due to hypothalamic GRF deficiency rather than to a deficiency of GH.

An additional finding was that Somatomedin C levels increased in most patients in spite of an unremarkable increase in GH blood titers. Somatomedin C is generally considered to be the substance that actually causes growth in most tissues, and it appears to be produced in response to the amount of circulating GH. Why Somatomedin C is increased more than GH in response to GRF is unknown. GRF is not known to cause Somatomedin C production directly, although this possibility must be considered.

In another study of GRF-40, four patients who were on therapy to correct GH deficiency were tested with GRF. In these still growing children, GH titers in the blood were increased much more dramatically than those described above. Although these four patients had been on GH therapy, they were not receiving the hormone at the time of the study.

This work shows that GRF may be a substitute for therapy in some children with hypopituitary short stature. Since the molecule is shorter than hGH (44 amino acids vs. 194 amino acids) it is more easily synthesized, and may be made available for future clinical use.

Rat Growth Hormone Gene Implanted into Mouse Eggs

In an attempt to introduce genes from one mammalian species into another, rat growth hormone (GH) gene has been incorporated into fertilized mouse eggs.

The rat GH gene from which the 5' regulatory region had been deleted, was fused to a mouse Metallothionein-I gene promoter. This was done in order to activate the GH gene when heavy metals are included in the diet of the recipient animals. The fused gene was then incorporated into bacterial plasmids, which in turn, were injected into the male pronucleus of newly fertilized mouse eggs. These fertilized eggs containing the injected genes were allowed to divide several times. Then the eggs were transplanted into the uteri of foster mothers made pseudopregnant by hormone injection. Offspring born from this procedure were weaned at normal age and then fed normal solid diet. Their drinking water, however, was supplemented with small amounts of zinc sulfate which was added to activate the Metallothionein-I gene promoter attached to the rat GH gene fragment. Results of this experiment show that the GH gene from the rat was activated in the mice and produced GH, since all mice in which a rat GH gene was shown to have survived grew up to two (2) times as large as their litter mate controls. One male mouse was able to pass on the enhanced growth tendency to offspring, indicating that at least in this case, the new gene was incorporated into the genome of this male mouse.

The results of this experiment raise the possibility that similar methods migh be applied to commercially valuable animals, to increase meat production or shorten growing times. The technique might also be valuable in the study or cure of certain genetic diseases, where replacement of a gene may alleviate severe symptoms. Finally, since these mice had very high GH levels in the blood, insertion of genes for various peptides might raise levels high enough so that animal "factories" could be developed in which the recipient would manufacture large amounts of hormones which could be harvested from the blood.

Localization of a Gene Sequence Responsible for Progesterone Regulation of an Ovalbumin Gene

Recent studies have shown that the region 5' of the steroid responsive genes is important for steroid regulation. A group of investigators have constructed fused genes containing varying lengths of the 5' flanking region, the first few base pairs of the ovalbumin gene and the structural region of the chicken beta globin genes. Up to 373 base pairs of the 5' flanking region of the ovalbumin were included in these fused genes. The hybrid genes were then inserted into a plasmid and used to infect chick oviduct cells in culture. A key feature of these hybrid genes is that the ovalbumin promoter (and possible regulatory sequences) are intact and respond *in vitro* to the effects of progesterone.

With the complete hybrid gene, progesterone elicited an eight-fold induction of the fused gene transcripts in the cultured cells. When hybrid genes containing deletions were used however, the results were different. Deletion of sequences

5' to base pair -95 (where base pair 0 is the first base pair of the ovalbumin gene) elminated almost all the steroid mediated transcripts, while deletion to -222 allowed almost normal levels of transcription to take place. These results suggest that those sequences between -95 and -222 in the ovalbumin flanking region are needed for maximal physiological expression. More importantly, purified receptor has been shown to bind to a region between -195 and -150 in such a way as to alter the normal pattern of this DNA when treated with a DNA degrading enzyme. This region houses a sequence, $\mathrm{AAAAT}^{G}_{T}\mathrm{G}^{G}_{A}\mathrm{C}$, which is also present in six other hormone regulatable egg white protein genes and occurs at about position -140 in most of these genes. This suggests that there is a common pathway and perhaps a common receptor binding area for egg white genes.

Metabolic Diseases Research Program

The primary objectives of this program are to support investigator initiated studies of fundamental metabolic processes and basic and clinical studies of various inborn metabolic diseases (e.g., cystic fibrosis, lysosomal storage diseases, glycogen storage diseases, etc.). Major foci of basic research investigations include membrane structure, biological transport, and enzymatic mechanisms as they regulate normal and abnormal metabolic processes. Additional areas of emphasis include studies related to the diagnosis, etiology, pathogenesis and treatment of a large number of inborn metabolic diseases. Some highlights of recent research accomplishments are summarized below.

Direct Role of Membrane Lipids in Hormone and Neurotransmitter Receptor Function

Hormones bind to highly specific membrane receptors and initiate a number of biochemical processes. These processes result in translation of the hormonal message into physiologic phenomena such as muscle contraction, nerve impulses, glandular secretion, and others. Researchers, supported by the program, recently succeeded in delipidating (defatting) a neurohormone receptor in order to study the effects of lipids on its mechanism of action. The receptor was solubilized and extensively freed of phospholipids and cholesterol. The delipidated preparation showed only insignificant ability to bind the neurohormone substrate. When various lipids were added back to the delipidated receptor, substrate binding affinities were restored dependent on the type of lipid added. Some lipids were more effective than others in restoring receptor binding ability. When the relipidated receptor was placed into a functional cell membrane missing the receptor, it performed the tasks of the intact neurohormone receptor, namely that of activating a message transmitting system called the adenylate cyclase system.

These initial findings demonstrate in an eloquent and elegant fashion the dependence of hormone-receptor interaction on membrane lipids and help us understand the mechanism by which hormonal messages are transmitted. The role of these lipids could be compared to that of lubricants which facilitate free movement of joints. Since the adenylate cyclase system is one of the major hormone message transduction systems in the body, this understanding is essential for maintaining health, as well as recognizing and controlling disease.

Elucidating the Molecular Basis for HPRT Deficiency Disease

Inherited metabolic diseases are caused by gene mutations which result in a defective enzyme-protein unable to perform its function of catalyst for chemical reactions in the body. Thus, unwanted and unneeded chemical products, produced during vital metabolic processes, can accumulate in various organs. The physiolgic consequences of such deposits can be devastating on the body and result in lengthy sufferings and premature death. In the Lesch-Nyhan syndrome, due to a deficiency in the enzyme hypoxanthine-guanine phosphoribosyl-transferase (HPRT), activity of this enzyme is virtually absent. The disease is characterized by overproduction of uric acid, severe central nervous system disorders and a compulsive form of self-mutilation. In hereditary gout, HPRT activity is partially deficient, so that afflicted subjects accumulate uric acid, but do not suffer from neurologic abnormalities. It has been established that HPRT is coded by a single structural gene located on the X chromosome. Recently scientists isolated DNA sequences that are complementary to the messenger RNA, that codes for human HPRT. This resulted in identification of 90% of the nucleotide sequence of human HPRT mRNA, and subsequently led to the understanding of the HPRT gene structure. The native, fully active human enzyme has been isolated, purified and its amino acid sequence established. The amino acid sequence predicted from the nucleotide sequence of the coding region was identical to that defined by traditional protein-sequence analysis.

Based on the above, researchers supported by the program were able to establish the exact molecular changes that render the HPRT partially active or inactive in the two diseases described above. Thus, the research group purified, to apparent homogeneity, HPRT from erythrocytes and cultured lymphoblasts of five unrelated patients from five different locations, all with HPRT deficiency. $\text{HPRT}_{\text{Toronto}}$, $\text{HPRT}_{\text{London}}$, $\text{HPRT}_{\text{Ann Arbor}}$, and $\text{HPRT}_{\text{Munich}}$, were from patients wit gout, $\text{HPRT}_{\text{Kingston}}$ was from a patient with the Lesch-Nyhan syndrome. Each isolated mutant enzyme had a unique set of structual and functional abnormalities. By establishing the animo acid sequences of four of the five HPRT variants, the researchers have established that one amino acid in each variant was substituted by another amino acid. Each substitution occurred at a different location in the enzymes studied. Further studies showed differences in intracellular concentrations and in the activity parameters of the five variant enzymes. It is possible that all these differences are due to the amino acid substitutions discovered. Detailed analysis of the isolated HPRT variants indicates that HPRT deficiency is frequently caused by single base mutations in the gene that lead to deleterious amino acid substitutions in the expressed enzyme. In Lesch-Nyhan syndrome, a majority of the patients retain little, if any, enzyme protein. In gout, a majority of the apparent defects in gene expression are probably caused by gene mutations that either result in a labile enzyme variant, or affect the normal processing of HPRT mRNA. Elucidation of the heterogenous nature of these diseases and understanding their molecular biology and genetics will be instrumental in the development of new treatment modalities.

Identification of the Deficient Enzyme Responsible for Biotin Depletion in Affected Infants and Children

Vitamins are essential co-factors needed for the normal functioning of a great variety of enzymes. It is known that at least three carboxylase enzymes require biotin (vitamin H) for their normal functioning and that biotin is chemically attached to a specific amino acid for each enzyme. Until recently biotin dependent carboxylase deficiency, an autosomal recessive rare disease, was assumed to be due to a disorder in biotin metabolism or transport. Both neonatal and an early childhood onset form of this disease are know to exist and even though they are distinct syndromes, both forms are, in general, associated with an increase in body acidity, skin rash, absence of hair, failure of muscle coordination and developmental delay.

Recently, researchers showed that in the late onset form, the primary biochemical defect is a deficiency in biotinidase activity, rather than a defect in the intestinal absorption of biotin. Biotinidase is an enzyme essential for regeneration of free biotin from a complex with an amino acid moiety of the carboxylase enzymes. It is now clear that significantly reduced concentrations or complete absence of biotinidase will result in partial or complete depletion of biotin in the bodies of afflicted children. These children, being unable to recycle biotin, are entirely dependent on biotin supplementation. Indeed, administration of pharmacologic doses of biotin can lead to complete remission of symptoms by restoration of normal enzyme activity. The researchers also developed an assay for in vivo measurement of biotinidase. By using this assay in studying afflicted subjects the research group found that the neurologic or cutaneous symptoms are the first to appear, while increase in the level of body acids is a secondary manifestation of the disease.

New Approaches to the Design of Superactive and Superstable Synthetic Peptides

Peptide hormones and neurotransmitters are the primary agents of communication between different cell types in the human body. When the concentration or physico-chemical properties of these compounds are significantly modified, communication patterns and their resulting biochemical processes are modified and a disease state can develop. Therefore, analogs of natural peptide hormones and neurotransmitters with appropriate properties and activities have great potentials as drugs and pharmaceuticals. There are certain properties that successful peptide drugs should elicit: (1) high specificity for a single receptor type; (2) stability in biologic environment; (3) long lasting activity; (4) high potency; and, (5) activity through oral administration. In an effort to overcome major difficulties posed by some of the above criteria new drug design approaches were needed. One such promising approach is the use of restriction of peptide structure and conformation. Alpha-melanotropin (alpha-MSH) is a peptide hormone known to be responsible for skin darkening, melanin pigment production and most recently was found to be associated with fetal development, central nervous system functioning and thermoregulation. This peptide was utilized to crystallize the new approach to the design of superactive peptide homologs.

The research team identified chemical and biochemical transformation which produced mixtures with improved biologic activity. They isolated the most active compound and determined its chemical structure. By utilizing this information, the researchers proceeded to test which peptide fragment of the analog might retain the exceptional biologic potency previously identified. The most active fragment identified contained a D-phenylalanine (D-Phe) amino acid which replaced the natural L-phenylalanine (L-Phe) in the original alpha-MSH. This observation led to the conclusion that the D-Phe for L-Phe substitution could stabilize an active conformation at the receptor. A conformational model was designed, synthesized and tested for both biologic activity and resistance to degradation. Finally, it was found that a cyclic peptide analog with frozen conformation provided both superpotency and superstability. For example, at extremely low concentrations, the biologic activity of the synthetic "Super"-derivative was 10,000 times that of alpha-MSH, and it showed prolonged *in vivo* activity lasting for weeks.

Cystic Fibrosis (CF)

A major focus of research on the basic defect of CF has been on processes controlling the reabsorption (recovery) of salt through the ducts of the sweat glands and other epithelial tissues of CF patients. For several years it has generally been assumed that the observed aberrant rate of ion transport in CF patients was primarily due to a defect in the transport of sodium ions. However, new results indicate that an important contributor to this phenomenon is the relative impermeability of some CF membranes to the reabsorption of chloride ions. This selective impermeability has been found in the luminal membrane of the sweat duct and also in the nasal mucosa of CF patients.

If the lining of the tracheobronchial tract (of which nasal mucosa is a part) is generally impermeable to chloride, this impermeability could be an important cause of the respiratory deterioration which is ultimately fatal to most CF patients. Further research in this area could lead to important advances in our understanding of both the basic and clinical aspects of CF.

Several investigators continue their attempts to isolate and characterize a "CF factor" from the blood or other body fluids of CF patients. Studies published from time to time over the last 15 years have suggested that such a factor could be the primary cause of some or all of the phenomena associated with CF and could be closely linked to the defective gene(s) responsible for the disease. To date, the factor remains unidentified, and recent research indicates that some phenomena previously attributed to the "CF factor" (e.g., ciliary dyskinesia) can also be caused by fluids from normal individuals as well as of CF patients. These results, coupled with the continued failure of most investigators to convincingly isolate and characterize the factor, makes this once popular approach to CF appear less promising.

Since 1983, the Institute has supported a CF core center at Case Western Reserve University (CWRU). The CF group at CWRU has received continuous support from NIH for over 20 years, mainly through the program project mechanism, and is widely recognized as an outstanding center for clinical and basic research on this disease. The goal of the Core Center is to integrate, coordinate, and foster high quality, interdisciplinary research related to cystic fibrosis. This center conducts investigations on a diverse set of projects, all of which are aimed at elucidating the underlying mechanism(s) responsible for the many manifestations of CF, which include understanding the primary pathogenetic process, elucidate the pulmonary and exocrine gland pathophysiology, as well as to develop improved methods of management of this disease.

Diabetes in CF Patients

Approximately eight percent of CF patients have fasting hypoglycemia, apparently due to insulinopenia to all stimuli. This incidence of hyperglycemia in the CF population is over 20 times that in comparable control populations. Although the vascular complications characteristic of insulin-dependent diabetes are almost never observed in CF patients with hyperglycemia, recent evidence indicates that this may be due to the reduced survival times of CF patients rather than to an inherent difference in their disease process. All CF patients studied had normal fundi on ophthalmoscopy, fundus photographs, and fluorescein angiography. However, in a study of the eyes of CF patients, when the mean fluorescein concentration of the vitreous is measured, those patients with hyperglycemia showed higher levels of the dye than did patients with normal carbohydrate tolerance.

Moreover, the mean fluorescein concentration of the carbohydrate intolerant CF individuals did not differ significantly from that of sex and age-matched insulin-dependent diabetics without CF. These results indicate that one of the earliest detectable functional abnormalities of diabetes, believed to be associated with breakdown of the blood-retinal barrier, occurs in diabetes secondary to CF with a severity similar to that found in insulin-dependent diabetes. This, in turn, suggests that as the survival of CF patients increases, those with glucose intolerance may develop the same vascular complications as do other diabetic patients.

ANNUAL REPORT

DIVISION OF DIGESTIVE DISEASES AND NUTRITION

OFFICE OF THE DIVISION DIRECTOR

The Division of Digestive Diseases and Nutrition is responsible for the extramural support of grants, contracts, and training for research pertaining to normal functions, pathophysiology, and diseases and disorders of the gastrointestinal tract and associated organs and to basic science of nutrition and to clinical nutrition. The organs encompassed by the digestive disease program include the esophagus, stomach, small and large intestines, anorectum, liver, biliary tract and pancreas. Among the major digestive diseases are esophagitis, peptic ulcer, diverticulitis, infectious diarrhea, inflammatory bowel diseases, malabsorption, gallstones, cirrhosis, hepatitis, and pancreatitis. The known causes include infections, metabolic disorders, genetic defects, neuromuscular disorders, abnormal immune reactions, toxins, and reactions to life stresses. Nutrition research supported by the Division includes prevention and control of obesity, improved nutrition of hospitalized patients, normal dietary requirements and special requirements during disease stress and surgery, safe levels of nutrients, measurement of the state of a patient's nutrition, trace mineral nutrition, and enteral and parenteral nutrition.

Digestive diseases constitute a health problem of great magnitude. Digestive diseases account for 10% of the total economic burden of illness; 15% of all admissions to general hospitals; 12% of all admissions to Veterans Administration hospitals; and 25% of all surgical operations (excluding tonsillectomies). Th Division of Digestive Diseases and Nutrition continues to receive large numbe of meritorious applications for research and research training support and supports research in a broad area. A modest, but important, initiative in a center grant program has been implemented with a request for applications for exploratory center grants. These grants prepared awardee institutions to apply for core center grants. Other recent Requests for Applications have included the following areas and topics: Liver Transplantation Research; Preservation of the Donor Liver and Assessment of Function; Research on Anorectal Diseases and Disorders. A Request for Proposals was issued for a Liver Transplantation Data Base.

The Division has been guided in its research program for digestive diseases by the long-range plan proposed by the National Commission on Digestive Diseases, by the research report of the National Digestive Diseases Advisory Board, and by ad hoc advisory committees. In nutrition, it has also been guided by advisory committees.

The National Digestive Diseases Advisory Board was established by Public Law 94-538 and chartered on May 13, 1981. The Board is responsible for updating and implementing the long-range plan. The Board has presented testimony to authorization and appropriation committees of Congress. It has identified needs and opportunities for research in the digestive diseases field, and the need for (1) a center grants program, (2) an epidemiology and data systems program, (3) the National Digestive Diseases Information and Education Clearinghouse, and (4) the continuation of authority for the Board.

The six subcommittees of the Board continue to update and develop the recommendations and intent of the National Digestive Diseases Commission as expressed in the Commission report of 1977. The Centers Subcommittee addressed needs, issues, and policy related to implementation of a centers grant program in digestive diseases by the NIH. It assisted the NIADDK with the concepts and development of a request for applications for exploratory grants.

The Research Subcommittee convened the chairman of the research committees of professional digestive disease organizations. They identified 14 key areas and then selected 14 experts in gastrointestinal research to provide leadership in updating the Commission's research plan for these areas. A report on digestive diseases research advances and research opportunities was released in the fall of 1983.

The Education Subcommittee developed a national digestive diseases education program. This program includes support from volunteer health agencies, Federal agencies, and the private sector. It achieved support for, and completion of, a survey concerning public knowledge of attitudes towards digestive diseases conducted by Louis Harris and Associates. The program received support from a pharmaceutical firm for the development, production, and distribution of a brochure on digestive diseases.

The Epidemiology Subcommittee completed the consolidation of ICD 8 and ICD 9 codes for the classification of digestive diseases. The members completed a study of the ICD 9 listings and developed a comprehensive list of disease categories belonging to digestive diseases. This represents the first such listing for the field. Using these disease categories, the Subcommittee is reviewing Health Care Financing Administration data (1980 MEDPAR file) on a 20% sample of discharges of Medicare beneficiaries.

The Patient Care Subcommittee planned and prepared a conference on the application of decision analysis to the problems of digestive diseases. This conference reviewed major elements in decision analysis and applied them to the development of a program for surveillance of individuals with a familial history of cancer of the colon.

ESOPHAGEAL, GASTRIC AND COLONIC DISEASES PROGRAM

The Esophageal, Gastric and Colonic Diseases Program is responsible for the extramural support of grant and contract awards in these areas. The following is a sample of recent research advances.

A Better Understanding of Esophageal Function

The understanding of the basic physiology and pathology of the esophagus has closely paralleled advances in other fields of investigation. One can trace the events in the fields of neurophysiology and anatomy to observe how they have been applied to the study of GI physiology in general and esophageal physiology in particular. In addition, the identification of an animal model whose functional anatomy is similar to humans has contributed substantially to the accuracy of generalizations extrapolated from the animal studies to the human situation.

An investigative group supported by NIADDK (Behar et al.) has been assessing the neural control of the lower esophageal sphincter (LES) in the cat. This group has produced data which suggest that it is a excitatory neural pathway which helps to control the tension at the LES. This pathway involves nerve cells which secrete both acetylcholine- and epinephrine-like compounds. The nerve endings terminating at the LES muscle have a special type of receptor known as an alpha-adrenergic receptor which permits the nerve to stimulate the LES muscle. It is likely, therefore, that these adrenergic neurons somehow contribute to the LES tone or contribute to its regulation in balance with inhibitory neural influences.

Investigations have continued to describe and quantify additional components in the neural control of the LES. Using the feline model in vitro, studies have been conducted to examine further the role of vasoactive intestinal peptide (VIP) in the control of LES pressure. (N.B. VIP has been suggested to be an inhibitory transmitter causing relaxation of the LES). Biancani et al (1982) have prepared antiserum to VIP and have tested the effect of this serum on the relaxation properties of LES muscle strips. In the strips which were treated with the anti-VIP serum, LES relaxation to a variety of relaxing stimuli was inhibited in varying degrees dependent upon the magnitude of the relaxing stimulus (electrical or VIP).

Other investigators in Boston have confirmed the findings that VIP is an inhibitor of LES pressure (Rattan et al.) and have also discovered that the esophgeal body, a region of the esophagus in which peristaltic contractions occur, is stimulated by VIP to cause contractions. These data support the concept that VIP is an inhibitory neurotransmitter helping to regulate LES pressure while serving as an excitatory transmitter for peristalsis in the esophageal body.

In addition Rattan and Goyal have described a number of opiod receptors on the opossum LES. Their studies suggest that (1) mu,. kappa, sigma and delta opiod receptors are present in the LES; (2) mu and kappa receptors are present on the sphincter muscle, and their activation causes inhibition of LES tone; (3) sigma receptor activation causes LES contraction by activition of cholinergic neurons; and (4) activation of delta receptors, which may lie on the sphincter muscle, causes LES contraction.

In an attempt to further understand the excitation-contraction coupling phenomena which regulates the tension of the LES muscle fibers, Biancani and Behar have conducted in vitro experiments on cat LES muscle strips (Excitation-contraction coupling can be defined as the aggregate of processes which permit an arriving nerve impulse to stimulate its effector structure, in this case the LES muscle fiber). By doing a variety of experiments with calcium solutions, the investigators observed that two sources of calcium have an impact on LES pressure. The first important source is extracellular or that bathing the cells. Intermittent or phasic contractions appear to depend on the extracellular source. However, the longer or tonic contractions seem to rely on stores of calcium which are found inside the cells. These data are consistent with earlier findings on the role of calcium on LES contraction.

Clinical studies at the Medical College of Wisconsin (Dodds et al.) have examined the mechanisms of gastroesophageal reflux in patients with esophagitis and controls. The investigators observed that the patients had significantly more episodes of reflux and had a lower LES pressure than controls. In the control subjects 94% of the reflux episodes were caused by transient sphincter relaxation. In the patient group 65% of the episodes of reflux were found to be accompanied by transient sphincter relaxation, 17% were associated with a fleeting increase in intra-abdominal pressure, and 18% occurred as a spontaneous reflux. For any one patient there was a large degree of variability in which reflux mechanism was dominant.

Further investigations by the same team (Dent *et al.*) have sought to determine whether there is a relationship between the normal cyclical contractions of the stomach and the pressure of the LES. Increased gastric contractions would increase the pressure on the gastric contents and tend to cause esophageal reflux if the LES tone was diminished or did not provide increased resistance to the increased intragastric (or intrabdominal) pressure. Using individuals who were asymptomatic for esophagitis (i.e., control subjects), the investigators measured the LES contractions during the active gastric contraction phases and observed that LES contractions were increased so as to maintain the pressure barrier between the gastric contents and the esophagus. In these normal individuals there were no episodes of gastroesophageal reflux caused by the normal gastric contractions.

Advances in Gastric and Parietal Cell Physiology: Basic Biology of Gastric Cells

Many studies are being conducted across the country to investigate the basic biology of gastric cells, with cell separation techniques which permit a relatively homogeneous culture of one type of cell to be grown *in vitro*. Such research has produced a number of interesting findings. Investigators at Emory University have been examining the basic metabolic and structural characteristics of these cultured cell suspensions (Hersey *et al.*; Gilbert and Hersey).

Studies of histamine metabolism have shown that at physiological concentrations of histidine, blockage of histidine transport may limit histamine synthesis in the intact cell and that measurement of histidine decarboxylase activity in tissue homogenates or cell extract may not reflect actual histidine decarboxylase activity *in vivo* (Beaven *et al.*). Further histidine decarboxylase activity is found only in the intact cell and is lost upon cell disruption (Beaven *et al.*).

Investigators at the University of California in San Francisco have studied the gastric tissue of fetal animals in order to determine if a model could be developed by which tissue culture could be used to study the development of stomach (Logsdon *et al.*). Using fetal rabbits, tissue was cultured on floating collagen cells and reactivity and viability determined. The investigators observed the formation of tight junctions between the cells with the cells containing an extensive endoplasmic reticular network. Electrical characteristics were studied and the ability of the tissue to respond to various gastric stimulants was assessed. The authors concluded that the culture system they described may allow the study of oxyntic cell differentiation and the development of H+, Na+ and Cl- transport in the gastric mucosa.

Similar techniques of cell isolation have been used extensively by Soll to study parietal cell function. Recent studies have dealt with the potentiating effects of gastric stimulants on isolated canine parietal cells. Studying the inhibitory actions of cimetidine, Soll has suggested that the potency of cimetidine in inhibiting all stimulants of acid secretion *in vivo* may be explained by the observation that histamine is an essential component of potentiating interactions that determine the parietal cell response to other stimulants.

The investigations on histamine and its analogues have already led to significant developments which produced two important drugs for the treatment of duodenal and gastric ulcer.

Advances in Gastric and Parietal Cell Physiology: Gastric Biology and Ulcer Research

Investigators at Harvard Medical School and the Beth Israel Hospital have been studying the effect of noxious agents on the surface epithelium of the stomach. Their work describes the time course of events following gastric injury. During the six hours after damage, there was a gradual process of restitution of epithelial integrity beginning with squamous-shaped cells that appeared to be migrating from the glands and ultimately concluding with complete epithelialization by cuboidal and columnar cells. Typical junctions complexes were present between adjacent epithelial cells. Reepithelialization was so uniform in the different preparations that, by examining the toluidine blue-stained sections in a blended fashion, it was possible to determine how long after injury the tissue was fixed. These observations indicated that there was a very intimate correlation between the restoration of epithelial continuity and the reestablishment of secretory and electrical activity under the experimental conditions (Svanes et al). Extending these studies, Rutten and Ito (1983) investigated the reconstitution of the electrical integrity of the guinea pig gastric mucosa damaged by hyperosmolar saline. Using standard *in vitro* techni they observed the following: (1) exposure of the guinea pig gastric mucosae to hypertonic saline on the luminal side will primarily result in surface cell epithelial cell destruction with an immediate drop in the transepithelial electrical values; and (2) after return to isotonic saline the damaged mucosa can repair itself within minutes, which then allows the reestablishment of the transepithelial electrical parameters within two hours. This repair process has been shown to be calcium dependent (Critchhow *et al*.).

Because of the previously reported observations that prostaglandins could protect the gastric mucosa from injury induced by such noxious stimuli as hyperosmolar NaCl, Svanes *et al*. used the above described method to determine whether prostaglandins might have the ability to prevent the epithelial changes or somehow alter the time course of those changes. Their microscopic studies confirmed that the prostaglandins did not protect the surface cells nor alter the reconstitution of the epithelium. Further, although 1 M NaCl injury causes the release of endogenous prostaglandins, neither the inhibition of formation of endogenous prostaglandins nor the superimposition of exogenous prostaglandins affects this injury or its repair. Using mucosal potential difference and net cationic flux as end points, the studies of Ligumsky *et al*. tend to support the observations of Svanes *et al*.

Aspirin has long been known to have adverse effects on the gastric mucosa. Large doses of aspirin, used by arthritics as anti-inflammatory agents, have produced gastritis and ulceration. Investigations are continuing to determine the mechanism by which aspirin induces these gastric lesions. Aspirin has been known to interfere with prostaglandin (PG) biosynthesis, and at the macroscopic level (in contrast to the microscopic level described in Svanes et al., Rutten and Ito,) PGs have been shown to protect the gastic mucosa from gross ulceration. Studies have continued to examine the enzymes involved in endogenous prostaglandin synthesis (Ligumsky et al.).

Another group of investigators at Harvard has been investigating agents which are capable of inducing duodenal ulcers. Structure-activity relationships were qualitatively and quantitatively examined for 56 chemicals (e.g., derivatives of propionitrile, acrylonitrile and cysteamine) which caused duodenal ulcer and/or adrenocortical necrosis in rats (Szabo et al.). Ulcerogenic activity was observed to be most intense in the carbonitriles attached to two or three carbon backbones and diminished by shortening, lengthening, branching, unsaturating, halogenating or hydroxylating the carbon chains. The authors suggest that chemical ulcerogens may interfere with the synthesis and/or binding to receptors of neurotransmitters such as gamma amino butyric acid and dopamine. A structural similarity between two carbon ulcerogens and the ethylamine side chain of dopamine and histamine was also apparent. In additional experiments the group demonstrated an inhibition by ergot alkaloids or apomorphine of the duodenal ulcers induced by cysteamine or propionitrile. A single dose of bromocriptine or lergotrile decreased the elevated gastric output after the administration of the duodenal ulcerogens. It was also shown that receptors existed in the rat duodenal and gastric mucosa and muscularis propria for specific ^{3}Hdopamine binding sites and a dopamine-sensitive adenylate cyclase (possible D-1 receptors) which were sensitive to treatment with duodenal ulcerogens (Szabo et al.).

INTESTINAL AND PANCREATIC DISEASES PROGRAM

Although a considerable amount of research and other activities, both basic and clinical, either has been reported by grantees or initiated by the program during the past year, only a few examples are cited to illustrate progress in some areas of interest.

Mediation of Enterotoxin and Microbial Adherence in Infectious Diarrheas by Host Mucosal Receptors

Very little progress has been made in defining the specific receptors on the gastrointestinal surface of the host which mediate adherence. Evidence was presented that these are genetically determined, age-related, and that they vary at different levels of the gastrointestinal tract, thus providing a molecular basis for understanding levels of colonization, age-related onset of diseases, and resistant and susceptible host populations. Host receptors might be present in the intestinal mucous gel (a layer of complex glycolipids and glycoproteins covering the epithelial cells of the intestinal lining) or on intrinsic membrane glycoproteins or glycolipids. It is now well established that the glycolipid, GM_1 ganglioside, is a specific receptor for cholera toxin. The toxin exerts its effect after first binding to the oligosaccharide moiety of this ganglioside on the surface of the cell. The strict specificity of the receptor structure for the binding of cholera toxin is indicated by the fact that when N-acetylneuraminic acid is removed from or added to GM_1 ganglioside, a substantial reduction in the binding affinity occurs. Recently, evidence has been presented indicating that rat intestinal microvillus membrane glycoproteins may also bind to cholera toxin. However, the relative pathophysiological significance of glycolipid and glycoprotein receptors for cholera toxin has yet to be described.

It has been known for many years that certain strains of *E. coli* can agglutinate red blood cells and that agglutination can be inhibited by D-mannose. Subsequent investigation revealed that certain strains of *E. coli* have on their surfaces lectin-like molecules (fimbriae) which bind to mannose receptors on epithelial cells. Another strain of *E. coli*, possessing fimbriae which consist of the antigen called K-88, produces a severe and often fatal diarrhea in neonatal pigs. This strain of *E. coli* appears to bind via the fimbriae to porcine intestinal cell surface glycoconjugates, particularly those with terminal N-acetylgalactosamine or N-acetylglucosamine residues in the carbohydrate side chains. In the K99 system toxigenic to bovine species, the receptor appears to be GM_2 ganglioside.

With regard to parasitic diseases, adhesion of trophozoites of *Entamoeba histolytica* to monolayer cultures of human intestinal cell lines has been shown to be mediated by a carbohydrate-binding protein (lectin) present on the trophozoites. The *in vitro* interaction of amoebae with intestinal cells in this system could be inhibited by either purified ameoba lectin or by analogous plant lectins. A giardiasis lectin responsible for hemagglutination has also been described.

Adhesion for some pathogens may be more complicated than a simple attachment of bacterial pili to receptors on mucosal cells, requiring, in addition, penetration of the mucous gel barrier before they can reach the cells. Moreover, only *V. cholerae* bacteria are capable of penetrating the mucous gel, and it appears that, to be able to penetrate the gel and colonize the intestine, they must also be attracted by, and follow the gradient of, an as yet unidentified chemic Bacterial adhesion to epithelial cell surfaces, therefore, represents only the final step of several leading to association with the mucosa. Consequently there may be a number of points at which it is possible to interfere with the early stages of infections. All in all, the research on pili and bacterial adhesion has provided some promising new leads for prevention of a wide range of important bacterial diseases. Thus, protection against infectious diarrheas may be possible either by immunizing with the bacterial pili or by developing appropriate mucosal surface receptor site analogs to either prevent colonization or dislodge the causative microorganisms from the intestinal mucosal surface of the host.

Two Genetic Loci Control the Murine Immune Response to A-gliadin, a Wheat Protein that Activates Gluten-Sensitive Enteropathy

Gluten-sensitive enteropathy (GSE, Celiac Sprue) is a disease that affects the small intestinal mucosa and results in the malabsorption of most nutrients. The disease in children and adults is associated with the ingestion of dietary gluten. Oral gluten challenge of gluten-sensitive individuals results in a loss of the normal small intestinal villous architecture (villous atrophy), most strikingly in the proximal small bowel (i.e., jejunum). GSE is known to be activated by protein(s) in the gliadin faction of wheat gluten and similar proteins in rye and barley. Gliadin, obtained by ethanol extraction of wheat gluten, contains at least 40 components, which can be assigned on the basis of electrophoretic mobilities to 4 major groups (i.e., α, β, γ, and ω), all rich in the amino acids glutamine and proline. Bernardin *et al*. purified an α-

gliadin fraction, that was later called A-gliadin, and which has been well characterized. This fraction has been shown to precipitate the lesion of GSE in susceptible individuals. How gliadin(s) result in epithelial cell damage and villous atrophy is unknown. However, one of the striking features of GSE is the infiltration of the jejunal lamina propria with plasma cells and the epithelial cell layer with lymphocytes. There are reports also of increased mucosal antibody synthesis to a variety of dietary antigens and components of gluten in patients with GSE. Finally, the major histocompatibility complex (MHC) alleles HLA-B8, -Dw3, and -DRw3 occur at high frequency in patients with GSE. The HLA-D region is now recognized to be analogous to the I-region of H-2, the murine MHC, which is well established to be involved in immunoregulation. Although such indirect evidence may support an immunologic mechanism as important in the pathogenesis of GSE, little is known regarding the nature, regulation, and specificity of the immune response to the gliadin proteins that cause disease.

Dr. Kagnoff et al. at the University of California at San Diego have recently examined the genetic control of the murine humoral immune response to purified A-gliadin. Studies with congenic and noncongenic inbred strains of mice, using a radioimmunoassay (measuring IgG anti-A-gliadin antibody levels) and a plaque-forming cell assay developed in their laboratory, revealed that two separate genetic regions determine the murine T-dependent antibody response to A-gliadin: H-2 on chromosome 17 and the immunoglobulin heavy chain (C_H) allotype locus (Igh) on chromosome 12. These findings in mice establish a clear linkage between the MHC and the regulation of the immune response to a protein capable of causing human disease, suggesting that MHC-coded immune response genes may control T-dependent humoral antibody and T cell-mediated immune responses to dietary gliadin in man. Although more than 80% of patients in certain populations with GSE have the MHC HLA-B8, Dw3 and DRw3 alleles, many individuals with these same HLA alleles ingest dietary gluten and do not develop disease. Further, family studies indicate that HLA-identical siblings are often disparate for the development of GSE. Such observations have led to the suggestion that a second genetic region may be important in the pathogenesis of this disease. The above studies with A-gliadin in mice suggest that the second genetic locus may be linked to the genes that code for immunoglobulin heavy chain allotype. Preliminary studies in man indicate that this is indeed the case. These observations and suggestions are in agreement with the speculation that GSE might be characterized by a disturbance in the balance between helper and suppressor influences due to an immune response gene defect mainfesting as an abnormal tendency to respond positively to oral challenge with gliadin.

LIVER AND BILIARY TRACT DISEASES PROGRAM

The program supports studies of the structure, function and diseases of the liver and biliary tract. Disease entities include inflammatory, metabolic and genetic diseases of the liver such as hepatitis, cirrhosis, Wilson's disease, primary biliary cirrhosis, fatty liver, hepatic encephalopathy, Dubin-Johnson syndrome and Gilbert's disease. It also includes studies on liver regeneration, liver assist and liver transplantation as well as liver ischemia, portal hypertension and toxic liver disorders. The bilary tract and its diseases include cholestasis, cholesterol and pigment gallstones, and bile salt, bile pigment and cholesterol metabolism.

The Liver and Biliary Diseases Program utilizes many funding mechanisms to implement the program, including regular research grants, program projects and centers, career development awards, clinical investigators awards, new investigator research awards, training grants, fellowship awards and contracts. A sampling of research follows.

Liver Cell Heterogenity and Functional Implications

A conceptual advance has occured in viewing hepatocytes as heterogeneous in respect to structure, enzyme content and function. In the past, functions of the liver such as the uptake, metabolism, synthesis and biliary secretion of compounds have generally been studied either at the level of the entire organ or at the intracellular level. The liver cell, however, functions within the environment of a microvascular unit, the hepatic acinus, which provides conditions for cell heterogeneity.

The distribution of blood flow is such that the progressive removal of solutes between the acinar inflow via the hepatic arteriole and portal venule and the acinar outlet via the hepatic venule, generates concentration gradients in the blood and in the hepatocyte. In addition, intrinsic cellular differences of a quantitative nature are seen in the hepatocytes located near the inlet and near the outlet. For example, cells near the inlet (zone 1) have a higher concentration of phosphoenolpyruvate carboxykinase, a rate limiting enzyme in gluconeogenesis. Hepatocytes near the outlet (zone 3) have higher concentrations of mixed-function oxidases which metabolize many drugs. Zone 1 hepatocytes have a greater capacity to transport bile salts at physiological concentrations; zone 3 hepatocytes show an earlier induction of smooth endoplasmic reticulum and of drug metabolizing enzymes when phenobarbital is administered.

Methodological advances have allowed separation and analysis of zones of liver cells by microdissection and by gradient separations according to densities or volumes. Labelling by fluorescent compounds may further simplify separation by means of fluorescence-activated cell sorters. Lipid soluble fluorescein diacetate and two water-soluble positively charged molecules (acridine orange and rhodamine B) label acinar zone 1, whereas water-soluble negatively charged fluorescein isothiocyanate, labels acinar zone 3.

Micropuncture of the Intact Liver Cell and of the Bile Canaliculus

Using cultured rat liver cells in which two hepatocytes join together to form a closed bile canaliculus, an NIADDK grantee together with a colleague from the University of Vienna, has succeeded in micropuncturing both the liver cell and the bile canaliculus. They have recorded the electrical potential in the cells and in the canaliculus for the first time in these very small cells. Bile secretion and dilation of canalicular vacuoles was stimulated by administering taurocholic acid and then 34 cells and their canalicular vacuoles were punctured with glass microelectrodes at a flat angle under visual control at 25oC. Measurements were made as the electrode first touched the cell membrane (small positive deflection of the potential and increase in resistance), punctured the cell (negative potential of about -20 mV), punctured the canalicular vacuole (positive displacement) and finally impaled the second liver cell. The input resistance of the biliary space was lower than the resistance of the liver cells (33 vs 79 M ohm). Therefore, extensive passive ion permeation occurs between biliary spaces and the bathing solution along a paracellular shunt pathway existing in parallel with passive and perhaps active transport components across the less permeable liver cell membranes.

The other very recent advance connected with this was the demonstration of contractions of the bile canaliculus using time-lapse cinephotomicrography. Living hepatocytes were observed under standard methods of cell isolation and culture. The contractions are regular, canalicular contraction time lasts 60 seconds and the usual interval between contractions is 5 1/2 minutes. The finding of a regular pattern of contraction suggests an ordered programmed and integrated mechanism which entails a high degree of communication and functional coordination between neighboring hepatocytes.

Micropuncture of the bile canaliculus will probably open up the field of understanding of bile secretion in the same manner as micropuncture of the renal tubule caused an explosion in the understanding of renal physiology. Investigators have tried for the past 20-30 years to gain insights into the events going on at the very first step in the secretion of bile and occurring at the most minute level of the biliary tree, the canaliculus. In the past, stop-flow analysis, attempts at micro puncture, etc. have all met with disappointing results and although events further down the biliary tree could be explored, those at the the canaliculus could not be because of the formidable technical problems.

NUTRITION PROGRAM

Introduction

The Nutrition Program, NIADDK, has continued to foster and support research and training in the broad areas of fundamental and clinical nutrition in order to advance knowledge concerning 1) the function and requirements of nutrients, 2) the relationship of diet (and nutrients) to health and disease, and 3) the prevention and treatment of diseases through the application of nutrition research. The fundamental research supported by the Nutrition Program generally has been nutrient-centered rather than focused on a particular disease, organ or life cycle state, while the clinical investigations usually are related to problems affecting the nutritional status of the whole individual, such as obesity, parenteral nutrition, anorexia nervosa and combined nutrient-drug management of patients. Discoveries from basic research have advanced our knowledge of the function of essential dietary nutrients in the whole body and have found wide application in clinical medicine.

The common occurrence of nutrition-related diseases or conditions such as obesity, diabetes, osteoporosis, alcoholism, anemia and atherosclerosis in segments of the U.S. population attests to the need for a better understanding of the role of nutrients in these clinical problems. The greatest promise for solutions to such problems lies in fundamental research on the mechanism of action of specific nutritional factors, and nutrient interactions, as well as the role of diet or nutrients as metabolic regulators, as physiological modifiers of behavior. Recent technological advances, including mass spectrometry (using stable isotopes), high-pressure liquid chromotography, radioimmunoassay and rapid analysis of amino acids and trace metals, offer promise for rapid progress in such basic nutrient-focused studies of importance in human health.

The nutrition program continued to stress the following priority research area

1) Fundamental studies on the metabolic role of specific dietary components, including essential amino acids, protein, vitamins, minerals, essential fatty acids, fats, carbohydrates and metabolized energy.

2) Investigation of the underlying causes of obesity, with the aim of developing appropriate methods for classification, prevention and control; and studies on dietary and physiological factors that affect eating behavior, thermogenesis and proliferation of adipocyte numbers.

3) Studies on nutritional requirements in health and disease with emphasis on individual and environmental differences.

4) Studies on the nutritional support of the patient as it affects nutritional status of the whole individual with such conditions as obesity, surgical trauma, burns, or chronic renal failure; and opportunities for nutritional research in patients sustained for long periods on TPN.

5) Basic studies on the role of "dietary fiber" on transit time, digestion, rate of absorption, intestinal microflora, and interactions with nutrients, drugs, bile salts, and other substances.

6) Research on nutrition and infection and immune competence.

A significant amount of nutrition related research was supported by other NIADDK programs, especially Digestive Diseases, Diabetes, Metabolic Diseases, Hematology, Kidney Diseases, Endocrinology and Musculoskelatal Diseases progra

Clinical Nutrition Research Unit (CNRUs)

The Nutrition Program supports five of the seven CNRUs presently funded by NIH. These are located at:

1) University of Chicago (Dr. Irwin Rosenberg)

2) University of Wisconsin (Dr. Alfred Harper)

3) Vanderbilt University (Dr. Harry Greene)

4) Medical College of Georgia (Dr. Elaine Feldman)

5) Columbia University (Dr. Myron Winick)

Research in human nutrition is truly interdisciplinary and complex, being dependent on the close interactions of several basic research disciplines for breakthrough in fundamental knowledge and on the appropriate medical specialities for rapid integration into clinical studies. Through the CNRU mechanism, the NIH has made great strides toward upgrading the role of clinical nutrition in the participating institutions. The CNRU program has helped to initiate and improve multidisciplinary research projects in clinical nutrition, recruit new investigators to the field and strengthen training environments.

A report on the evaluation of the CNRUs has been prepared in collaboration with NCI. Emphasis on shared core laboratory facilities has proven especially valuable in increasing the number of multidisciplinary studies. An average of 23 new clinical research protocols, ranging from 7 to 40, are now active at each CNRU. Studies include the role of nutrition in diabetes, cystic fibrosis, digestive diseases, renal disease, cardiovascular disease, cancer, and care of seriously ill patients as well as growth, aging and metabolism. Improvements also have resulted in the coordination and delivery of nutrition-related patient care, as well as in communication of nutrition research advances to professionals and to the public. Education of medical studies in clinical nutrition also has been strengthened as a result of the CNRUs.

The New Investigator awards, supported temporarily with CNRU funds, are expected to yield a meaningful number of well-trained clinical research scientists in the near future.

The fourth annual meeting of the NCRU directors was held January 9 and 10, 1984, in New York City. The CNRU at Columbia University served as host of the first day, and the CNRU at Memorial Sloan-Kettering Cancer Center (supported by NCI), the second. The meeting provided a forum to exchange information and pursue opportunities for institutional collaboration in the areas of laboratory methodology, educational activities, and clinical research.

Program Announcements

The multi-institute program announcement entitled "Studies on Overnutrition and Obesity," was published in the NIH Guide for Grants and Contracts, March 30, 1984. This was done collaboratively with nine other institutes through the NIH Nutrition Coordinating Committee. The Nutrition Program Director, NIADDK, helped to lead this effort.

The Nutrition Program averages approximately 62 competing research applications (AM primary) per year which relate to obesity, of which about 20 are funded.

Currently, the multi-institute announcement for "Core Grants for Clinical Nutrition Research Units," published in the NIH Guide for Grants and Contracts, January 26, 1979, is being updated and revised. Coordination is being handled through the NIH Nutrition Coordinating Committee.

Workshops

In FY 84, the Nutrition Program provided partial support and/or participated in the following workshops or conferences:

1) FASEB Summary Research Conference on Vitamin A and retinoids, July 1984.

2) Vitamin A and Cancer Prevention Sponsored by the U.S. and Japanese Malnutrition Panels, U.S.-Japan Cooperative Medical Sciences Program (NIADDK) and NCI, February 28-29, 1984.

3) A workshop on structured lipids and their use in clinical nutrition has been planned for September 1984.

U.S.-Japan Malnutrition Panel

NIADDK has continued to administer the U.S. Malnutrition Panel of the U.S.-Japan Cooperative Medical Science Program. The research fostered under the U.S. and Japanese Malnutrition program is targeted primarily at those problems and solutions which most benefit the undernourished of Asia. The most common states of undernutrition are protein-energy malnutrition and iron and vitamin A deficiencies. Superimposed are various disease states, particularly diarrheal infections, which cause impaired utilization and loss of nutrients and constitute the primary cause of death of children under 5 years of age. Iron deficiency, which is widespread, is believed to interfere with immune response, cause lowered resistance to infection and reduce the functional physical capabilities and possibly impair mental and learning processes. Vitamin A deficiency in turn causes many thousands of cases of damaged vision and total blindness in young children each year.

The annual joint meeting of the U.S. and Japanese Malnutrition Panels was held February 27, 1984, in Bethesda, MD. Following the joint meeting, a two-day workshop was held on "Vitamin A and Cancer Prevention" which was co-sponsored by the U.S.-Japan Malnutrition Panel in association with the NIADDK and the National Cancer Institute. The meeting focused on epidemiologic and clinical studies related to vitamin A and its possible role in cancer risk. The conference was quite well attended. The amount of interest shown and the discussion on the subjects presented supported the impression that the conference served a useful purpose of bringing together and stimulating exchange among people from the more basic sciences with epidemiological and clinical researchers. The presentations are to be published as a journal supplement and will be made available upon request.

Finally, it was tentatively decided that the next joint meeting would be held in Japan in early December 1984 with the symposium being on the topic "Calcium Metabolism and Aging."

Research by Contract

The second year of a two-year completion contract with the Food and Nutrition Board, National Academy of Sciences is being renewed This contract, initiated December 15, 1979, is entitled "Preparation of the Tenth Edition of Recommended Dietary Allowances and Related Studies." The revised recommended dietary allowances (RDAs) are to be completed in 1985. Guidelines for the use of the RDAs are to be prepared for publication at the same time. These activities are proceeding on schedule. The committee on dietary allowances and its subcommittee on uses of the RDAs have held several working meetings using the committee-study and mini-workshop approach. A revised draft report has been prepared on guidelines for uses of the RDAs.

Vitamin D Metabolite Increases Trabecular Bone Volume, Improves Calcium Retention and Decreases Bone Fracture Rate in Postmenopausal Osteoporotic Women

The discovery in recent years of hydroxylated derivatives of vitamin D has led to new concepts of calcium homeostasis. The liver converts the vitamin to the plasma transport form (25-hydroxycholecalciferol) and finally the kidney converts the plasma form to the hormone (1,25 dihydroxycholecaliferol) which acts to express the function of the vitamin. Target tissues responsive to this active form include intestinal mucosa, bone, kidney, parathyroid gland, pancreas, pituitary and others. These basic discoveries have led to renewed interest in the role of vitamin D in calcium and phosphorus transport and retention in bone, and its relationship to bone disease states, especially osteodystrophy of renal disease and osteoporosis.

Dr. Larry Riggs at the Mayo Clinic, Dr. J. C. Gallagher and Dr. Hector DeLuca and others, of the University of Wisconsin, have found that as little as 0.5 micrograms of 1,25 dihydroxycholecalciferol administered daily to postmenopausal women with osteoporosis not only increased calcium balances but, more importantly, caused an increase in trabecular bone volume and decreased bone facture rate. This group also found that plasma 1,25 dihydroxy-cholecalciferol levels fall after age 50, and especially in postmenopausal women. Estradial treatment of postmenopausal women caused a 30-40% elevation in circulating levels of 1,25 dihydroxycholecalciferol and corresponding increases in calcium absorption.

Japanese scientists have completed a clinical trial using 1-alphahydroxycholecalciferol for treatment of postmenopausal osteoporosis with similar results, since the alpha hydroxy form is readily converted into the active 1,25 dihydroxy active form by the liver. A clinical trial also is underway at four locations in the U.S.

The Wisconsin researchers also have synthesized 24,24 difloro-1,25-dihydroxycholecalciferol and found the difluro substituted compound to be 10-100 times more active due to longer lasting biologically activity.

Current knowledge about the endocrine function of vitamin D3 metabolites suggests many metabolic implications as well as applications to a number of human disease states. The vitamin D metabolites (25-hydroxycholecalciferol, 1,25-dihydroxycholecalciferol, and 1-alphahydroxycholicalciferol) have been isolated, identified and chemically characterized under NIADDK support. These are currently being used for the treatment of a wide variety of metabolic bone diseases, for example, renal osteodystrophy, hypoparathyroidism, pseudohypoparathyroidism, vitamin D resistant rickets, and now osteoporosis. Furthermore, the measurement of vitamin D metabolites are being used in diagnosis of disease. Thus, low plasma levels of 25-hydroxycholecalciferol indicate intestinal malabsorption, biliary secretion failure, or poor vitamin D nutrition. Reduced 1,25-dyhydroxycholecalciferol in conjunction with parythyroid hormone, blood phosphorus and blood calicum levels, have been useful in diagnosing a variety of diseases. There can be little doubt that the study of the metabolism and function of vitamin D has had marked physiological significance.

Trace Minerals in Total Parenteral Nutrition

Total parenteral nutrition (TPN) is a special means of providing certain patients with the proper amounts of the essential dietary nutrients by intravenous administration for the maintenance of health. TPN may be required for extensive periods in patients with (1) malabsorption for the intestinal tract, (2) extensive gastrointestinal surgery, or (3) hypermetabolic state continuing for a considerable time, such as in patients with extensive burns. In a patient unable to ingest, digest or absorb nutrients and not fed parenterally, starvation results in negative nitrogen balance and wasting of both muscle and viscera. This ultimately leads to dysfunction of the liver, muscle and immune system. Loss of 35 to 40 of lean body mass during such an illness usually results in death.

Much progress has been made during the past decade in the development of TPN mixtures and systems that provide appropriate levels of amino acids, vitamins and electrolytes. However, much remains unknown about the rate of depletion of essential trace minerals and levels needed by patients maintained on TPN for indefinite periods.

New information on requirements and reports of trace element deficiencies in patients fed by TPN has led to an increased awareness of the importance of providing proper levels of trace elements in TPN solutions, particularly when the parenteral route is the sole or primary source of nutrients for prolonged periods. The trace elements in addition to iron and iodine, now recognized as essential in man include zinc, copper, chromium, and selenium as a result of the deficiences recognized in patients on TPN.

In addition Abumrad *et al*., 1981, have described a molybdenum deficiency in a patient with prolonged TPN therapy. Other trace minerals which may be needed for long term TPN, but those for which little information is available, are manganese, vanadium and silicon. The American Medical Association published guidelines for essential trace element preparations for parenteral use in 1979, and has recently convened specialist to update these guidelines.

The ability to provide nutrients by parenteral means in amounts needed to maintain health of patients who cannot ingest, digest, or absorb food, represents a major technological advance. Not only is TPN extremely important in rehabilitation of the critically ill or surgically stressed, or in other short-term conditions w food cannot be taken enterally, but the number of individuals requiring long-t TPN in order to live is over 1,000 and this number is doubling each year.

A Rapid Test of Dark Adaptation

The classical dark adaptation test is a tedious, lengthy (45 minute) procedure requiring cumbersome and expensive instrumentation making it unsuitable for use under field conditions. Serum levels of vitamin A vary over a wide range in subjects with functional deficiency and thus have little predictive accuracy in identifying subclinical deficiency states.

A rapid method for testing dark adaptation which requires a minimum of transportable equipment has recently been described and is suitable for evaluation of rod function. However, certain inconsistencies were noted when compared to results obtained with classical dark adaptation testing on a Goldman Weekers dark adaptometer. Vinton and Russell have described a modified rapid dark adaptation test based on the method of Thorton. The major change introduced involved "matching" the color discs used in the test to cones. That is, the intensities of the discs were matched to cone function (not to rod function) so that under dim light conditions, where cones cannot discriminate color, the ability to separate the discs by brightness is dependent on rod function. It is based on a shift of retinal wavelength sensitivity as the eye dark adapts so that blue appears brighter than red when rods begin to activate under darkened lighting conditions. The subject separates sequentially the white, then blue disc from a pile of red, white and blue discs. The procedure is timed until 100% accuracy of sorting is achieved.

Evaluation of this modified rapid test of dark adaptation is 32 healthy subjects and 25 patients, 14 of whom were vitamin A deficient, revealed a sensitivity of 95% (1.5 s. d.) and a specificity of 91%. This modified rapid test (under 6 minutes) is suitable for field use. The test has been used satisfactorily with children ranging in age from 4 to 13 years under field conditions in Guatemala and Baltimore. The mean time required for the test was 2.8 minutes.

Zinc deficiency, presumably due to impairment in vitamin A transport, also may delay dark adaptation. Zinc supplementation has been found to correct impaired dark adaptation in adults not responsive to vitamin A therapy, suggesting that this method may be useful in identifying zinc deficiency as well.

A high prevalence of vitamin A deficiency has been demonstrated in patients with biliary cirrhosis and pancreatic insufficiency. However, in patients with protein calorie malnutrition, the impaired dark adaptation resulting from vitamin A deficiency is uncorrectable with vitamin A and zinc administration until protein is repleted.

The earliest clinical manifestation of a vitamin A deficiency is a decrease in dark adaptation ability or night vision. Early detection of hypovitaminosis A and institution of prophylactic therapy could dramatically reduce the prevalence of xerophthalmia and vitamin A deficiency blindness among populations at risk. Vitamin A deficiency is a major cause of preventable blindness, contributing to 20,000 to 100,000 new cases annually throughout the world. Deficiencies of vitamin A, zinc and protein frequently may coexist. With a simplified rapid test of dark adaptation as described and validated, quantitative determination of rod function can become assessable in any clinical situation. For example, this test also can be used as a diagnostic tool for identifying low zinc status or other condition (protein deficiency) in which rod function in the retina is impaired.

NATIONAL DIGESTIVE DISEASES EDUCATION AND INFORMATION CLEARINGHOUSE

The National Digestive Diseases Education and Information Clearinghouse (NDDEIC) is an information service of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (NIADDK). The Clearinghouse acts as the coordinator of a national effort to educate patients, their families, physicians and other health care providers, and the public about the prevention, early detection, and management of digestive diseases.

The Clearinghouse provides a central point for the exchange of information among professional organizations, foundations, and voluntary health organizations involved with digestive health and diseases; aids with the distribution of available education materials, and identifies needed information and educational materials, and encourages production of these needed materials.

In 1983 the Clearinghouse was reviewed by a Public Health Service Panel and a report from this panel listed the accomplishments of the Clearinghouse. The report listed as a significant accomplishment the Clearinghouse's role in fostering the coalescence of the diverse professional and lay organizations in supporting a national education and information effort in digestive diseases. Also the Panel Report recommended development of a long range plan; definition of the primary targeted audiences for the Clearinghouse and the information the audiences were to receive; revision of the process for the development of fact sheets, and the development of an evaluation plan.

Two meetings were held this year with the advisors to the Clearinghouse in which the Review Panel's recommendations were addressed. An advisors' planning committee was formed to undertake the task of developing a long range plan for the Clearinghouse. A process is under development designed to shorten the production time of the fact sheets.

Five fact sheets were published. These are: "Facts and Fallacies About Digestive Diseases," "Dyspepsia," "Irritable Bowel Syndrome," and "What You Should Know About Stomach Ulcers." Other fact sheets under development include "Common Digestive Disorders," "Your Digestive System: How It Works and What It Does," "What You Should Know About Inflammatory Bowel Disease," "X-rays and Ultrasound: Diagnostic Techniques for Digestive Diseases," "Alcohol and Digestion," "Smoking and Digestion," "Malabsorption and Maldigestion," and "Yellow Baby." Other notes and publications under development include "What Is Hyperacidity?," "Research Update: New Drugs for Control of Gastric Acid Hypersecretion," and "Peptic Ulcers and Surgery." In cooperation with the National Institute on Aging, two Age Page publications are under development: "Aging and the Digestive System," and "Comfort and Your Digestive System."

The "Directory of Digestive Diseases Organizations" was updated and a bibliography of patient education materials in digestive diseases was produced as well as a listing of audiovisual materials in digestive diseases. A "Letter from the Clearinghouse" was developed and distributed. Two listings of Digestive Diseases materials that have been identified were produced. These are "Digestive Diseases Patient Education Materials" and "Digestive Diseases Health Professional Mater[illegible]

The Clearinghouse had exhibits at eight professional meetings and one health fair: Patient Education in the Primary Care Setting, September 12-13, 1983, at Kansas City, MO; Parklawn Health Fair, September 22-23, 1983, at Rockville, MD; American Medical Writers Association Conference, November 9-12, 1983, at Philadelphia, PA; Geriatric Drug Use: Clinical and Social Perspectives, February 27-28, 1984, at Washington, D.C.; National Rural Primary Care Conference, March 18-21, 1984, at Roanoke, VA; Digestive Diseases Week, May 20-23, 1984, at New Orleans, LA; and Patients Education from the Professional Perspective Workshop, June 9, 1984, at Washington, D.C.

A plan for designing a combined patient education database with an accompanying thesaurus was developed by seven DHHS clearinghouses and information centers including the NDDEIC. The purpose of the database is to combine references and full text of short information materials from all seven information programs. Users of the database will have access to information from all seven information programs. Testing of the design is being carried out by three of the clearinghouses. A pilot testing of the database was carried out with the cooperation of the National Library of Medicine, the National Health Information Clearinghouse, and the V.A. Library. A thesaurus is under development for the Digestive Diseases Clearinghouse.

Distribution of digestive diseases materials continues to be primarily through professional and lay organizations. The Clearinghouse collaborated with the lay digestive diseases organizations for distribution of material to their chapters and members. These Clearinghouse education materials included the fact sheets, "Cirrhosis of the Liver," "Diarrhea: Infectious and Other Causes," "Heartburn," "Gallstone Disease," "Bleeding in the Digestive Tract," "Milk Intolerance," "Constipation," "Facts and Fallacies About Digestive Diseases," and "Dyspepsia," as well as a flyer, "Director of Organizations," the "Glossary of Digestive Diseases Terms," "Notes," and the "Letter" from the Clearinghouse.

The Clearinghouse participated in the National Digestive Diseases Education Program with the National Digestive Diseases Advisory Board's Subcommittee on Education and with the Coalition of Digestive Diseases Organizations, an organization of many of the lay and professional digestive diseases societies. Two meetings of the *ad hoc* steering committee were held.

ANNUAL REPORT

DIVISION OF KIDNEY, UROLOGIC AND HEMATOLOGIC DISEASES

The Division of Kidney, Urologic and Hematologic Diseases supports and administers research in a broad range of basic and clinical sciences. Continual assessment of the programs of the Division is based on liaison with the extramural scientists, including ad hoc advisory committees. This evaluation process allows the Division to facilitate support mechanisms for important new areas of research which are most responsive to the needs of the research community and ultimately to patients with kidney, urinary tract and hematologic diseases.

Two major trans-NIH reports pertaining to chronic renal disease were prepared in the past year. The NIH Coordinating Committee for Chronic Renal Disease submitted its report to the Director of the NIH regarding the current level of research support and outlining the new opportunities which can be brought to bear in the prevention and treatment of conditions causing chronic renal diseases. A report of the Public Health Service Coordinating Committee on End Stage Renal Disease has also been submitted which included recommendations for approaches to facilitate clinical data collection from patients with end stage renal disease, activities for prevention of certain chronic renal diseases, identification of fruitful areas of research into the causes of end stage renal disease, as well as treatment of end stage renal disease by transplantation and dialysis.

Efforts have begun to develop a clinical trial to evaluate the impact of dietary protein restriction on the progression of chronic renal disease. Initiatives in this area have been prompted by the scientific reports, including uncontrolled clinical observations, which suggest that protein restriction can attenuate the maladaptive compensatory mechanisms which normally emerge following nephron injury. The proposed study would also test whether the need for maintenance dialysis therapy can be delayed, and thereby reduced, by dietary manipulation. The Division of Kidney, Urologic and Hematologic Diseases has issued an RFA for a cooperative agreement entitled, "Collaborative Clinical Study of Dietary Modification on the Course of Progressive Renal Disease". Applications have been reviewed and it is anticipated that six participating clinical centers and a data coordinating center will begin preparation in the fall of 1984 of a uniform protocol to be utilized by all centers. A pilot clinical study is expected to begin in mid-1985.

The Division has recognized the underdevelopment of immunological research pertaining to the pathogenesis and treatment of chronic renal diseases. This concern is based on the recognition that the majority of diseases causing end stage renal failure appear to involve immunological mechanisms. A Program Announcement soliciting research grant applications for studies on the immune basis of renal disease has been released which has resulted in new activities in this area.

The urology program has solicited extensive input from leaders in multiple disciplines to identify new research opportunities and to develop new initatives for research in the area of urinary tract structure, function and diseases. Planning has also begun for a Division sponsored, multidisciplinary conference on Benign Prostatic Hypertrophy in 1985.

The Division supports a broad base of research in hematologic diseases, particularly emphasizing red blood cell disorders, hematopoiesis, immunohematology, as well as research in hemoglobinopathies, nutritional anemias and iron metabolism, among others. Planning for research support in the area of hematologic diseases has been facilitated by the publication in 1983 of the study "Research Needs in Hematology."

The Division sponsored two widely attended conferences at the NIH in 1984. A Consensus Development Conference on Analgesic Associated Kidney Disease was held in February 1984. A formal list of recommendations regarding the uses and risks of analgesic and anti-pyretic compounds has been produced. A Conference on the Use of Monoclonal Antibodies in Renal Research was held at NIH in May 1984. A rich interchange of information was shared among participants from industry, technology, basic and clinical immunology, and the nephrology community in regard to the use of monoclonal antibodies in kidney research.

RENAL PHYSIOLOGY/PATHOPHYSIOLOGY PROGRAM

The Renal Physiology/Pathophysiology Program of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases continues to support research on the structure and function of the kidney, including metabolic studies, transport and fluid-electrolyte dynamics; diseases and mechanisms leading to chronic renal disease, such as immunologically mediated glomerular and systemic diseases, including glomerulonephritis, interstitial and lupus nephritis; polycystic kidney disease; and effects of drugs, nephrotoxins and environmental toxins on the kidney. The following are highlights of some of the research activities supported by the Renal Physiology/Pathophysiology Program.

The Role of Mesangium in Health and Disease

Diseases causing mesangial expansion including membranoproliferative glomerulonephritis and diabetes account for very significant morbidity, mortality and social cost from progressive renal disease. The mechanisms by which these and other disorders, characterized by marked expansion of the mesangium, lead to proteinuria, hypertension and renal failure are largely unknown.

Supporting the hypothesis that clinical manifestations, like proteinuria and hypertension in some glomerular diseases involves the mesangium, Dr. Alfred Michael and colleagues have developed a rat model which exhibits marked mesangial expansion when animals are administered polyvinyl alcohols (PVA). This results in the appearance of monocytic cells in the mesangium, alterations in the mesangial uptake and processing of macromolecules and glomerular basement membrane (GBM) thickening. In recent studies utilizing the model in which a kidney is transplanted from a rat with mesangial PVA localization to a normal rat, this group has documented that GBM thickening is greatest in those peripheral glomerular capillary walls contiguous to mesangial areas most markedly expanded by PVA. In the earlier stages the PVA localization is variable from one glomerulus to another and from tuft to tuft within a given glomerulus, but with time, the mesangial lesion in this model becomes more uniform. They have also observed the appearance of a progressive albuminuria in this model at the time of PVA injection and continuing one month following the injection. Furthermore, this albuminuria has its onset while the animals are still normotensive. Light microscopic observations suggest that mesangial expansion in the PVA-Munich-Wis rats along with the observed proteinuria is analogous to that seen in humans with diabetic nephropathy and who have significant albuminuria (in excess of 400 mg/24 hours). These findings therefore raise significant implications regarding the pathogenetic factors associated with glomerular dysfunction in diseases where the mesangium is altered, such as diabetes mellitus. Although these preliminary observations in rats are semiquantitative and subjective, it is of interest to note that these investigators' light microscopic estimates of mesangial thickening are very similar to their results with electron microscopic morphometric measurements of changes in the mesangial area in human diabetic nephropathy. Thus measurements of the area of the mesangium, capillary lumen and peripheral capillary filtering surface in PVA rats should prove to be helpful in elucidating pathogenetic events which have relevance to human diseases associated with mesangial expansion.

A New Model of Human Membranous Nephropathy

Data presented by Border and colleagues is consistent with the hypothesis that cationic antigens induce glomerular immune complex deposition by directly binding to the glomerular basement membrane through electrostatic interaction and thus predispose to *in situ* immune complex formation. In contrast, anionic antigens seem to require the formation of circulating immune complexes which then deposit in the glomerular capillary wall. To test their hypothesis, they studied the differential effects of the polycation, protamine sulfate, and the immunosuppressive drug, methylprednisolone. The rationale for using protamine sulfate is that it is a weakly immunogenic, highly cationic and freely filtered molecule. Therefore it would be expected to bind to the anionic sites in the glomerulus and protect from the subsequent binding of a positive charged immuno Their *in vitro* experiments showed that when protamine was incubated with isolat glomerular basement membrane it subsequently inhibited the binding of cationic bovine serum albumin (BSA). When cationized BSA was administered to rabbits, the antigen was observed to bind to the outer portion of the glomerular basement membrane, where antibody to this antigen subsequently deposited to form immune aggregates *in situ*. In some of the animals that developed subepithelial immune deposits, the continued administration of protamine led to their disappearance and restoration of the normal structure of the glomerular capillary wall. This is reminiscent of the ability of high doses of free antigen to dissolve immune complex deposits as demonstrated in serum sickness nephritis caused by the administration of normal anionic BSA. The fact that subepithelial deposits can be readily dissolved is a new observation. The experimental evidence indicates that protamine's therapeutic effect appears to be mediated by its electrical charge rather than through any of its other pharmacologic properties. These observations are of potential importance in unravelling the pathogenetic events associated with the development of immune mediated glomerular injury and may lead to relevant new therapeutic approaches.

In related studies at the University of Minnesota, Vernier, *et al*. have developed a method that permits the demonstration, quantitation and cytochemical characterization by specific enzymatic digestion of the macromolecules responsible for the negatively charged sites in human glomeruli. They have analyzed the distribution of these sites in kidney specimens from five children with the congenital nephrotic syndrome, as compared with the distribution in normal subjects. Their findings demonstrated the presence of heparan sulfate-associated anion sites in the normal human glomerulus similar to that described in the rat and dog. They further report a reduction in the number of such sites in the lamina rara externa of five patients with a relatively rare cause of proteinuria - the congenital nephrotic syndrome. It is suggested that heparan-rich anionic sites fail to develop in the glomerular basement membrane and that this defect may be responsible for the proteinuria in this disorder.

NMR Imaging: Its Potential as a Research and Clinical Tool

Reports on the clinical application of nuclear magnetic resonance (NMR) have shown that the greatest strides have been made in the neurological and hematological fields, largely due to the fact that most of the experience has been gained in these areas. Nevertheless, results of basic investigations focusing on measurements of intracellular pH, ATP phosphate concentration and inorganic phosphate in rat kidney tissue and in the rectal gland of the spiny dogfish, *Squalus acanthias*, have supplied quantitative information about the role of ATP and phosphocreatine turnover during secretion by these two biological systems. NMR has also been used to monitor intracellular sodium, phosphocreatine and ATP in the same living frog skin. These and future results, which shed light on the sequential changes in intracellular sodium that accompany large increases in transepithelial transport in simple systems should lay the groundwork for the application of NMR technology to study non-invasively the cellular transport processes in whole organs, such as the kidney.

UROLOGY PROGRAM

The Urology Program supports research and training on benign prostatic hyperplasia, urinary tract infection, urolithiasis, obstructive uropathy, neuromuscular dysfunction of the urinary tract, vesicoureteral and intrarenal reflux, and sundry conditions such as Peyronie's disease, impotence, congenital anomalies of the lower urinary tract, etc. Progress in research, training and program development in several of these areas is reported below.

Urinary Tract: Reflux and Hydronephrosis

A number of physiological processes interact in the transport of urine from the kidney, storage in the bladder and micturition. Morphologic changes seriously restricting urine transport and micturition may cause significant reflux creating hydronephrosis. The hydrostatic pressure of the blocked urine in the kidney if left untreated, may cause progressive renal scarring and eventual loss of function. Reflux may occur in the absence or presence of infection. Reflux may occur in the fetus requiring *in utero* surgery if both ureters are blocked. In young children vesicoureteral reflux may be so serious as to require medical intervention to prevent progressive kidney scarring, loss of kidney function and development of hypertension. The most efficacious treatment intervention for vesicoureteral reflux is yet to be determined in prospective clinical trials, although, several less definitive efforts (Birmingham Study Group,; Hospital for Sick Children Trials) question the assumption that surgical treatment is superior to non-surgical treatment. Several advances, however, have been made during the past year.

- Using an animal model (lamb) investigators have developed surgical techniques resolving hydronephrosis *in utero*.

- Adenosine triphosphate (ATP) may function as a neurotransmitter in regulati urethra sphincteric control and (or) bladder contractions in the cat.

- The International Study on Reflux in children, which compares medical and surgical management of reflux, has actually achieved the status of an international effort with the combining of the United States and European studies this past year.

Research efforts will continue in an effort to pinpoint the precise causes of kidney damage following reflux, including the role of the infectious processes involved. Data have not been conclusive on the most efficacious treatment for vesicoureteral reflux in children, and the International Study offers the best hope to provide such definitive treatment information.

Benign Prostatic Hyperplasia

BPH is characterized by excessive growth of the prostate gland, and is associated with retention of urine and disruption of normal voiding. The morbidity and estimated medical costs (exceeding one billion annually) associated with BPH, make this disease a significant national health problem. Weighted averages computed on the basis of published studies clearly show that BPH is present in the younger as well as older male and is prevalent among males in Western, Asian and African countries. When these findings are considered with those showing the 5-10% of all prostatic specimens obtained from surgery for BPH show microscopic evidence of adenocarcinoma, it is clear that prostate research is an important national health area warranting intense investigation.

- Prostatic stromal tissue capable of inducing growth of the prostate has a greater amount of nuclear androgen (dihydrotestosterone) receptors than do prostatic epithelial nuclei.

- The nuclei of tissue taken from patients with BPH have higher concentrations of androgen receptors (dihydrotestosterone) than nuclei taken from normal prostates, whereas, total prostate gland contents of androgen are the same for BPH and normal glands.

- BPH tissue shows a significant reduction in converting androgen (dihydrotestosterone) responsible for abnormal prostatic growth to a biologically inactive form when compared to normal prostatic tissue.

- A major proportion of steroid binding capacity of nuclei from prostate tissue is contained in the nuclear matrix which also serves to anchor certain DNA sequences and RNA processing.

The recent advances to help to increase understanding of the biochemical and hormonal events associated with abnormal growth of prostatic tissues, and provide the rationale for experimental drugs currently under development for non-surgical treatment of BPH. One such approach uses an inhibitor (17B-N, N-diethylcarbamoyl-4-aza-4-methyl-5a-androstan-3-one) of the enzyme which converts testosterone to the active androgen (dihydrotestosterone) responsible for growth of the prostate gland. The enzyme inhibitor has been shown to decrease prostatic volume in the beagle which, like man, naturally develops BPH. The close association in the nuclear matrix of bound steroid hormone and certain nucleic acids may offer more understanding in the future of hormone-gene interactions.

Urinary Tract Infection

Urinary tract infections are the second most common bacterial disease seen in the clinic, and are associated with considerable morbidity and loss of work time in affected individuals. Moreover, it is estimated that at least 8,000 deaths occur each year as result of infections related to the kidney. An especially pathogenic bacteria (E. coli 04H1) has been identified in the non-human primate as being responsible for pyelonephritis and research at the Karolinska Hospital in Sweden has identified similar bacteria in children suffering from pyelonephritis. The extent to which P-fimbriated bacteria are uniformly associated with pyelonephritis in the human has been disputed for not all children with such bacteria evidence infection. Other research in this area has concentrated on methods of combating infection by reducing surface adhesion of the invading bacteria, and by assessing autoimmune responses in animals genetically prone to early infection. The pathogenesis associated with P-fimbriated bacteria is of interest also to investigators interested in vesicoureteral reflux which often occurs in association with infection.

- Animals which die as a result of early infection have been found to develop a specific antibody against their own renal tubular cells; the initial immune response later damages the kidney tubules.

- Immunization of the vaginal mucosa in lower mammals has significantly reduced the adherence of bacteria to the urethra.

- Superoxide dismutase is being investigated as a preventative to renal scarring caused by chemotaxis resulting from bacterial induced inflammation.

Urolithiasis

Urolithiasis concerns the development of stones occurring primarily in the upper urinary tract (kidney; ureter) in industrialized countries. The major component of upper urinary tract stones is calcium, and there is lack of agreement on the extent to which excessive calcium present in the urine, a necessary condition for stone formation, results from pathophysiology of organic calcium balance, or genetic traits. The physicochemistry of stone formation in the kidney, and the role of naturally occurring inhibitors to stone formation in the urine continue to be investigated. There have been advances, however, in the medical treatment of absorptive hypercalciuria, and struvite (infectious) stones. Non-invasive destruction of kidney stones using shock-waves and techniques restricting the need for surgical intervention by use of the nephroscope, along with the medical treatments for stone dissolution and prevention suggest that therapeutic interventions may be progressing more rapidly than basic understanding of the dynamics of stone formation.

- FDA has approved a new drug (sodium cellulose phosphate) which may prevent stone recurrence in certain subjects by reducing intestinal absorption of calcium.

- FDA has approved a new drug (acetohydroxamic acid) which may be effective i preventing urease production which is responsible for creating the necessary pathological conditions leading to infectious stone (struvite) formation.

- Natural inhibitors for stone formation are found in the urine (e.g., citrate), and when combined with small radius ions (i.e., Fe++), inhibit stone crystallization more effectively than either the citrate or Fe++ alone.

CHRONIC RENAL DISEASE PROGRAM

The Chronic Renal Disease Program supports research in both, fundamental and clinical investigation of disease mechanisms responsible for end-stage renal disease, complications and, all aspects of treatment. There are three main subdivisions of the Program: Pathophysiology of Chronic Renal Failure, Renal Transplantation and Maintenance Therapies of End Stage Renal Disease.

Potential Beneficial Effects of Nitrogen-Free Analogues of Essential Amino Acids in the Treatment of Chronic Renal Disease May Exceed Those Produced by Essential Amino Acids

The basic principle for the management of renal insufficiency is the restriction of protein; in patients with chronic renal failure, the institution of a protein-poor diet results in improvement of the general condition, disappearance of uremic symptoms and a decrease in serum urea concentration. It seems clear that accumulation of nitrogenous metabolites play a major role in the pathogenesis of the uremic syndrome, but extreme care is warranted in restriction of dietary protein to avoid concomitant malnutrition. Several recent studies suggest that nutritional or metabolic factors may also affect the rate of progression of re disease. The evidence, although not conclusive, indicates that low protein diets, particularly when associated with careful control of phosphorus intake, may retard the rate of progression of renal failure in animals and humans.

Nitrogen free branched-chain keto analogues (BCKA) of essential amino acids have been experimentally utilized for treatment of chronic renal failure, liver insufficiency and certain genetic diseases, since these compounds lack the alpha-amino nitrogen and are rapidly converted to amino acids. Therefore, administering them may be almost tantamount to giving amino acids without increasing the nitrogen load. Information, nevertheless, is lacking on the possible in vivo role of BCKA in the inter-organ transport of branched-chain amino acids (BCAA); the in vivo oxidation and transamination of BCKA relative to BCAA; the tissue and bilogical fluids concentration; the hormonal regulation and influence of other agents on BCKA metabolization; the interplay with other amino acids and, the extent of utilization and incorporation of BCKA for protein synthesis. Nitrogen free BCKA can replace BCAA in the diet of animals and man though not with complete efficiency.

Over the past few years several investigators, independently, have developed methods designed to answer some of the questions outlined above. BCAA and BCKA metabolism in man, the conscious dog, and rats are being investigated by using isotopic and arterio-venous (A-V) difference techniques. While isotopic methods measure total body flux of substrates in and out of a central pool, the A-V difference technique measures the net contribution of various tissues to the flow of substrates from one organ to another. The combination of both techniques allow for measurements of the relative contributions of the different organs to total body flux. Preliminary data indicate that approximately 30% of the flux in the overnight fasted conscious dog occurs across the splachnic bed--half by the gut and half by the liver. Skeletal muscles produce BCAA in the post-absorptive period. The net flow of BCKA is from peripheral to visceral tissues. Therefore, the liver, gut, and kidneys equally utilize BCKA while skeletal muscles supply that amount. The dilution of label across the gut is due to an exchange of label for unlabeled BCAA molecules, while the dilution across the liver is partially (1/3) due to exchange and partially (2/3) to net addition of BCAA.

In another set of investigations to estimate the extent, as well as the relative rate of incorporation of BCKA into newly synthesized protein, ^{14}C-labelled BCKA and ^{3}H-labelled-BCAA were administered simultaneously intragastrically to normal rats and normal human volunteers. The mean incorporation ratio in protein of various rat organs varied from a high of 0.42 (in muscle) to a low of 0.22 (in liver). Whole body protein incorporation ratio averaged 0.30. In rats fed BCKA and a low protein diet for six days prior to isotope injection, whole body protein incorporation was unchanged. Mean incorporation in circulating proteins, such as isolated albumin, fibrinogen and IgG, was substantially higher than in whole mixed liver protein.

Normal volunteers were given the isotopes by constant intragastric infusion for six hours. Mean ratio of relative incorporation into plasma albumin (0.64) and fibrinogen (0.63) became constant after the third hour, and was indistinguishable from one another (this finding is consistent with the possibility that both proteins are synthesized from a common precursor pool of amino acids); in plasma IgG was 0.68; in red blood cell globin it was also constant, but higher (0.81), while in plasma leucine it averaged 0.62.

The results of these studies show that the extent, as well as rate of incorporation of labelled BCAA and BCKA into newly synthesized protein can be simultaneously quantitated in animals and man. There is a reduced nutritional efficiency of BCKA relative to BCAA and it is believed that enterally administered BCKA becomes incorporated into liver export proteins less efficiently (relative to BCAA) than it does into liver parenchymal proteins or peripherally synthesized proteins. The probability of a BCKA molecule becoming incorporated into newly formed protein depends on a series of complex steps such as: transport, oxidation, and transamination processes, protein synthesis and degradation in the organ where protein synthesis takes place as well as within the organ where it is measured.

Renal Allograft Survival Rates are Slightly Better in Cyclosporine Treated Renal Graft Recipients Than in Those Receiving Conventional Immunosuppression

As a result of a better understanding of histocompatibility systems, utilization of pre-transplant blood transfusions to condition potential renal transplant recipients, improved preservation techniques and better selection of patients, a decline in morbidity and mortality associated with renal transplantation has been observed in recent years. Overall graft survival has not really improved in the past decade, but we may be at a turning point in the history of transplantation, armed with new information about immunogenetics of rejection and new modes of therapy.

Cyclosporine (CsA) is a potent immunosuppressant agent used in the prevention of allograft rejection, which is derived from the fermentation broth of the fungus Trichoderma polysporum. Its immunosuppressive activity was first discovered by Borel et al. in Basel, Switzerland.

Subsequent extensive experimental studies in the rat, dog, rabbit, etc. revealed a strong immunosuppressive activity without bone-marrow suppression. Promising experimental results led Calne et al. (Cambridge, UK) to first initiate a clinical trial using CsA as the sole immunosuppressive agent in renal transplantation. Rejection prophylaxis did occur but an unexpectedly severe nephrotoxicity was responsible for a high incidence of early non-functioning grafts. Furthermore, the addition of other immunosuppressants to CsA led to an unacceptably high infection rate. Further clinical experience and the extension of these initial pilot studies to prospective randomized clinical trials with modifications of the original immunosuppressive protocols, provides the necessary scientific format for the ongoing objective evaluation of the efficacy, safety and possible toxic side-effects of CsA.

Increased interest and increased enthusiasm has evolved with the availability of CsA in the immunopharmacology armamentarium of renal transplantation.

Results in Clinical Trials

Since the initial trial by Calne et al. in 1978, every clinical series has demonstrated that CsA treatment induces graft survival at least as good as any other immunosuppressant regimen.

Discrepancies exist between published results obtained from collective data analysis derived from clinical studies conducted in European, Canadian, and American transplant centers. Some of these data indicate dramatic improvement in patient, as well as graft, survival rates with CsA treated patients compared to patients on standard immunosuppressive regimens. These data should be read and interpreted with extreme care since in the majority of cases a comparison is established between actual CsA treated patients versus either data retrospectively obtained from "historical controls" or derived from prospective randomized trials involving very different treatment protocols selected "individually" at each participating center for the standard-immunosuppression control group.

The interim analysis of an ongoing prospective randomized controlled clinical trial of CsA plus low dose steroids (prednisone) versus the standard immunosuppressive regimen of prednisone and azathioprine (AZA), plus antilymphocyte globulin (ALG) in the immediate post-transplant period demonstrates no significant difference in patient or graft survival rates when the two groups are compared. Actuarial overall patient and graft functional survival rates up to 26 months post-transplant, in all CsA and standard immunosuppression renal graft recipients, show that renal allograft survival rates are only slightly better (by 7% at one year) in CsA-prednisone than in AZA-prednisone-ALG treated renal allograft recipients. Patient survival rate is slightly better in the conventional treatment group (by 3% at one year). None of the differences between groups or in the subgroups, are statistically significant (all p values are > 0.1).

Some of the disadvantages associated with the use of CsA as opposed to the conventional regimens include: a longer duration of acute tubular necrosis -

if it occurs - in CsA treated patients, higher creatinine levels at all times post-transplant and more difficult management of hypertension and hyperkalemia (increased serum potassium levels). The advantages to the use of CsA include: fewer viral and bacterial infections, shorter hospitalization times, and fewer rejection episodes.

Therefore, even though it is possible for other trials to show a marked advantage of CsA over the conventional immunosuppressive regimen, it is important to realize that when certain features are incorporated into the standard immunosuppression regimen, such as patients being heavily transfused, splenectomized and receiving ALG, graft survival rates can be maximized.

As indicated earlier, clinical studies have shown CsA to be effective in the prevention of allograft rejection, but how this immunosuppressive effect manifests itself at the cellular and intercellular level is unknown; the metabolism and immunosuppressive action need further investigation as well. Further analysis will be necessary to determine which regimen is superior in terms of safety as well as efficacy for long-term maintenance of renal allograft function and which is the most cost-effective one.

HEMATOLOGY PROGRAM

The National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases supports hematologic research as a part of its mission to eliminate the threat of the chronic diseases that cause suffering, disability, and early death. Many of these chronic diseases exhibit manifestations which include anemia, caused by impaired blood production or faulty construction of the red blood cells. Anemias of chronic disease rank high among the clinical disorders that threaten lives or diminish the quality of life. The anemia of chronic renal disease occurs in most of the chronic renal disease patients treated annually in the U.S. Anemia is commonplace in patients with endocrine disorders, the collagen diseases, rheumatoid arthritis, ulcerative colitis and other digestive diseases, liver disorders, and a variety of inflammatory syndromes. These anemias, all of which are acquired secondary to chronic diseases, fall within the NIADDK responsibility, not only because of the basic hematologic involvement, but also because of the NIADDK mission related to renal disease, endocrine disorders, arthritis, and digestive and liver diseases.

Congenital, or hereditary anemias, generally resulting from some specific fixed disorder in body chemistry, and metabolism, or molecular design, also come within the sphere of the NIADDK Hematology Program. Such research support has led to a nearly complete analysis of normal and certain abnormal human hemoglobin gene sequences, and to safe and accurate techniques for prenatal diagnosis of sickle cell anemia and Cooley's anemia. Basic work on the hemoglobin molecule has been instrumental in establishing concepts in molecular disease, and today hemoglobin and hemoglobin diseases represent models for other genetic diseases.

Early Bone Marrow Transplantation in Childhood Acute Lymphoblastic Leukemia (ALL)

In the mid-1970s, bone marrow transplantation was considered as a tool for managing hematologic disease only in patients with far-advanced drug-resistant cases. Advances in chemotherapy of acute leukemia in children, and, more recently, adults, have allowed complete remissions in 95% of children with ALL, and in about 70% of adults. Some of the patients in remission relapsed on chemotherap or after chemotherapy was halted. These could not be saved until now. Recently, however, impressive progress has been achieved in bone marrow transplantation once a second remission has been obtained. Nearly all of these patients now can be cured. Early bone marrow transplantation in acute leukemia now cures the highest proportion of patients of any currently available treatment modality.

Bone marrow transplantation remains a technologically complex procedure, requiring currently, the availability of HLA-matched siblings. The high incidence of chemically-induced remissions now allows stabilization of disease in most ALL patients. Techniques have been developed to achieve destruction of the patient's leukemic cell population and immune system by chemotherapy and total body irradiation. Plasmapheresis may be used to circumvent ABO incompatibility, if present, or antibodies may be removed by insolubilized antigen or selective removal by a red blood cell separator. Following preparation of the patient, donor marrow is infused intravenously. These highly demanding procedures now can be accomplished with resulting engraftment into the recipient marrow. Survival of most of the good risk patients (those under 45 years of age, with HLA-matched siblings) has been reported, and relapses after two years of follow-up have been rare.

Bone marrow transplantation in patients with acute leukemia has been found to be a superior therapy in properly selected patients. Bone marrow transplantation thus represents a promising direction for the development of a complete therapy. Properly selected patients include primarily those under 45, who HLA-matched siblings.

Transformation of Cellular Genomes by Oncogenes, and Association with Hematologic and Other Neoplasms

Recent studies using gene transfer methodology have demonstrated the existence in human tumors of activated genes (oncogenes) which are capable of causing uncontrolled proliferation when transmitted to normal cells.

Cell transformation, proven to be a useful concept in hematologic research, has been found to occur in response to a variety of agents *in vitro* (chemical mutagens, viruses, and ionizing radiation). Convincing evidence now shows that RNA tumor viruses may transform cells by transferring RNA from malignant cells to normal ones. When the cellular genes, in association with control elements of the virus, are reintroduced into normal cells, tumors develop.

At the same time as these findings were made in viral research, newly-developed gene transfer techniques had been improved sufficiently to apply to these studies. DNA purified from donor cells was inserted into the nucleus of recipient cells, and was found to be incorporated into the genome. Some of this DNA was found to have the capacity to transform the recipient cells into malignant cells. The activity was transferrable, and, moreover, was subject to activation by a separable control segment of the DNA. This type of control segment, or transforming oncogene, has been detected in DNA preparations from a variety of malignancies including certain leukemias and lymphomas. Transforming genes recently have been found to be very similar in structure to RNA viral genes which cause cancer leading to speculation that they are related in origin.

Demonstration of the Mode of Inheritance of Hemochromatosis

Hemochromatosis, a progressive and fatal disorder if not recognized and treated at an early age, is an iron storage disease affecting a variety of body systems. In hemochromatosis, tissue iron overload is associated with skin pigmentation, liver cirrhosis, diabetes, and other abnormalities. Prior to recent studies, it was generally agreed that the disorder was inherited, but the mode of inheritance was disputed. The inability to resolve this question was complicated by the influence of other factors including age, sex, alcohol, iron intake, and iron loss.

The linkage of hemochromatosis to HLA genes on chromosome 6 has been established, and the mode of inheritance has been determined to be recessive. A major contribution towards resolving the mode of inheritance was the 1975 discovery of an association between specific histocompatibility antigens (HLA) of the human immune system, and hereditary hemochromatosis. The association with specific HLA antigens (mainly A3) was so strong that it was likely that the hereditary hemochromatosis gene and the HLA genes were present in the same chromosome, chromosome 6.

Studies have now been completed of 25 patient pedigrees, in which at least one individual with clinically apparent hemochromatosis has been detected. A recessive mode of inheritance was supported by: Pedigree studies in which unaffected parents produced multiple offspring with hemochromatosis, clinically evident hemochromatosis in 25% of siblings of individual with hemochromatosis (but in very few parents or offspring), and by an increased incidence of marriages between relatives among parents of affected individuals. The specific HLA genes linked to a hemochromatosis gene vary from pedigree to pedigree, but are consistent within a given pedigree.

Hemochromatosis is not rare, since in Utah and localities studied in France, 10-13% of the Caucasian population is heterozygous for the gene. Approximately 5 out of 1,000 individuals are homozygous. Full expression of the disease is found in many homozygous individuals. Recognition of the mode of inheritance should lead to a more frequent identification of homozygotes before onset of organ damage and end-stage disease, leading to prevention of serious illness. Improved genetic understanding can be coupled with the recent development of a technique of direct noninvasive magnetic measurements of hepatic iron stores, to determine precisely the degree of illness of patients, and should lead to the application of appropriate therapy.

A Rapid, Safe Prenatal Diagnostic Test for Sickle Cell Anemia and Thalassemia Has Been Developed

Prenatal diagnosis of sickle cell anemia, thalassemia, and other genetic disorders can be accomplished by direct DNA analysis of amniotic cells, and of first trimester transvaginal amniotic biopsies.

Enzymes (restriction enzymes) are available which cleave DNA at specific sites. After cleaving, the DNA fragments are separated by electrophoresis, immobilization on filters, and identified by hybridization with radioactive probes of complementary DNA. With this procedure, normal DNA yields distinct and reproducible patterns with each restriction enzyme. Some human gene mutations which cause genetic disorders, can be detected directly, by identifying an aberrant patter after restriction enzyme use, or indirectly, identifying a characteristic pattern which is linked to the mutated gene.

Direct analysis may be applied to sickle cell anemia, where the mutation abolishes a known restriction enzyme site. When normal DNA is digested with the enzyme, two fragments of 1.15 and 0.2 units (kilobases) in length are produced. The sickle mutation abolishes the cleavage site, and a fragment results which is 1.35 units long.

The indirect method has been used for β-thalassemia, since most mutations causing this disease do not involve alterations in restriction enzyme sites in the gene. However, the indirect method involves an extensive search for identifiable associated genetic polymorphisms, and is not practical as a widely available procedure. Recently, however, Dr. Y. W. Kan, of the University of California, San Francisco, who has pioneered much of the above technology, has prepared synthetic gene fragments to detect directly the mutation in the DNA that results in β-thalassemia. A synthetic probe for a thalassemia mutation binds only to the defective β-thalassemia DNA, while the synthetic probe for normal DNA binds only to the homozygous normal DNA under appropriate conditions. Both probes bind to a DNA fragment from heterozygous β-thalassemia. Thus it is possible to distinguish among the clinically normal, heterozygous (carrier), individuals and those homozygous for specific types of β-thalassemia.

Annual Report of the
Mathematical Research Branch
National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases

Current research projects of the Mathematical Research Branch reflect a broad range of interests in the development and application of theoretical models as well as quantitative methodologies to biological systems.

This research involves several different collaborations within the Branch and with other research groups, both at NIH and elsewhere. This report describes recent work in the areas of synaptic neurobiology, excitability and impulse propagation, electrical oscillations in nerve and secretory cells, microcirculation and facilitated transport, auditory physiology, molecular biology, and renal physiology.

Synaptic Neurobiology

Rall and Rinzel previously developed a biophysical theory to investigate the functional significance of dendritic spines as receivers of synaptic input. Under the assumption of passive membrane in the spine head they showed that by adjusting the value of the spine stem resistance R_{ss} the potency of a synaptic event could be altered effectively. Increasing R_{ss} leads to a monotonically decreasing somatic response; maximum sensitivity to changes in R_{ss} lies in the estimated phyiological range for some neurons. Recently we have considered the effect of active membrane in the spine head. For appropriate ranges of structural/electrical parameters we find that the post-synaptic response can be augmented by increasing R_{ss} through an intermediate (optimal) range of values. This is because the increased input resistance seen at the spine head causes a greater depolarization there and can thus bring the active membrane to threshold and deliver more current to the dendrite. If R_{ss} is too small, threshold is not surpassed and, of course, if R_{ss} is very large the voltage drop across R_{ss} is great and the dendritic response is small. Our results have been presented in several seminars and conference symposia; a paper has been submitted for publication. We are currently pursuing questions related to the firing of other active spine head membranes which are not directly activated by synapses but which might be brought to threshold by dendritic depolarization. Depending upon the density of spines and upon their membrane properties and sequence of activation one can imagine a chain reaction of spine head firings which propagates from one dendritic region to another. (Rall, Rinzel, Segev, Miller and colleague, Shepherd and colleagues).

For the multipolar neuron whose several dendritic trees have different values of L (the functional, electrotonic length), we have gone significantly beyond the analysis of decay transients and estimation of L-peel reported last year. We have an analytic solution which provides the complete EPSP shape produced by local (dendritic) injection of current proportional to $T \exp(-\alpha T)$. Computations for an example having one short tree ($L=0.8$) and three long trees ($L=1.8$) have been carried out and the resulting shape index plots (Peak Time versus Half Width) can be compared with those for the simpler reference case where all trees have the same L value. (Rall and Segev).

Excitability and Impulse Propagation.

The nonlinear cable equations for excitable membrane have traveling wave solutions which correspond to steady propagation of trains of nerve impulses. We have previously computed the dispersion relations, dependence of propagation speed c on interspike interval T, for such periodic solutions to various nerve conduction equations. We applied this relation in a formal kinematics to describe approximately the individual pulse trajectories during propagation. Currently we are exploiting the kinematics to guide and investigate conjectures about the outcome of pulse spacings during propagation based on particular but qualitative features of the disperion relation. (Rinzel and Maginu).

Steady propagation of excitation waves can be observed in some one-dimensional distributed excitable media such as the nerve axon and the Josephson junction line. Mathematically speaking, a solitary impulse wave in an excitable medium corresponds one-to-one to a homoclinic orbit of a certain dynamical system, and therefore the wave can be characterized qualitatively by using some topological properties of the homoclinic orbit. We introduced a topological characteristic (called the sign) of the solitary waves and showed that it is closely related to the bifurcation and the stability of the waves. By the use of this characteristic we can show, for example, the instability of the fast solitary wave on the Josephson junction line and the slow impulse wave on the nerve axon. (Maginu).

We can construct a periodic wavetrain of excitation waves by concatenating the solitary impulse waves periodically. This wavetrain is not always stable even if the individual waves are stable as solitary waves; namely, mutual interaction between adjacent waves can cause the instability of the periodic wavetrain. We introduced another topological characteristic (called the torsion) of the solitary impulse waves and showed that it is closely related to the stability of the concatenated wavetrains. By the use of this characteristic we can show, for example, that the periodic wavetrains of nerve impulses must be unstable if the impulse has an overshoot in its tail. (Maginu).

Usually an excitable medium has several qualitatively different periodic wavetrains according to the choice of its external parameters. We introduced two topological characteristics of periodic wavetrains and showed that they are closely related to bifurcations (simple bifurcation and period doubling bifurcation) and the stability of the wavetrains. The values of these characteristics can be determined uniquely if the dispersion relation curve (the propagation speed vs. the pulse interval) is known, and we can elucidate relationship between different types of wavetrains by using these characteristics. (Maginu).

The standard FitzHugh-Nagumo equation provides a qualitative description of nerve membrane excitability however it lacks certain identifiable physiological parameters. An alternate derivation of this simplified two-variable model retains greater biophysical realism and can reproduce, nearly quantitatively, many of the Hodgkin-Huxley model's phenomena. (Rinzel).

Electrical Oscillations in Neurons and Endocrine Cells.

Pancreatic β-cells (isolated islet preparation) exhibit a wide variety of electrical respnses to variations in glucose concentration. So does the Hodgkin-Huxley type model proposed by Chay and Keizer. We have explored the transition behavior as parameters are varied between the regimes of beating (continuous spiking) and bursting. At lower temperatures this transition is characterized by aperiodic responses; even though the model is deterministic the voltage oscillations may appear stochastic. Such dynamic phenomena are referred to as deterministic chaos and have been recently studied extensively in applications outside of excitable membrane biophysics. We have adapted theoretical concepts and methods from such contexts (e.g. from the work of Rinzel and Troy on chemical oscillators) to analyze the periodic and chaotic behavior of the Chay-Keizer model. A one-variable discrete-time representation of the dynaics provides an insightful way of accounting for much of the behavior. This simplified model is motivated biphysically by exploiting the significant role which intracellular calcium plays in regulating the spike activity; it describes the change in calcium from one spike to the next. (Chay and Rinzel).

Microcirculation and Facilitated Transport.

It has been shown experimentally that the presence of hemoglobin or myoglobin in an aqueous solution held in a slab with fixed oxygen concentrations on its sides increases the total flow of oxygen across the slab. We denote by facilitated transport F_f the difference between the flow of oxygen with and without the protein. Denote by x_1^0 and x_1^ℓ the oxygen concentrations at the high and low concentration sides of the transport path. The theoretical dependence of F_f on x_1^0 and x_1^ℓ, especially the decrement of F_f for large values of x_1^0 was discussed in the previous annual report.

The fact that for a given x_1^ℓ the flow F_f attains a maximum for some x_1^0 suggests a possible physiological significance. This was explored further by studying the transport associated with the hemoglobin body wall of the adult form of the Ascaris lumbricoides whose habitat is the relatively low oxygen concentration of the intestinal lumen of the host, and the myoglobin present in mammalian striated muscle. Let

$$\phi(x_1^\ell) = \max_{x_1^0} F_f(x_1^0, x_1^\ell) .$$

For the Ascaris the behavior of ϕ was compared with the behavior of the activity of the enzyme prolilhydroxylase of the parasite. It was found that for oxygen concentrations between 0 and 10 torr the decrease in the activity of the prolilhydroxylase was accompanied by an increase in ϕ.

For the striated muscle it was found that for oxygen concentrations between 0 and 30 torr the changes in the reduction state of the mitochondrial cytochrome c and in the ratio $[ATP]/([ADP]\cdot[P_i])$ in the cytosol were accompanied by parallel changes in ϕ.

To study the dependence on x_1^0 the following function is useful. Let

$$x_1^0 = X(x_1^\ell)$$

be defined as follows. For any x_1^ℓ, $X(x_1^\ell)$ is the oxygen concentration x_1^0 at which the maximum ϕ is attained by F_ℓ. Let A denote the interval of x_1^ℓ in which as x_1^ℓ decreases ϕ increases 70% of its maximum and let B denote the interval of x_1^0 defined by $x_1^0 = X(x_1^\ell)$ for x_1^ℓ in A.

For the Ascaris, A and B are the intervals $0 < x_1^\ell < 1.2$ torr and $4 < x_1^0 \leq 15$ torr respectively. The interval B correlate with the oxygen concentrations reported for the intestional lumen of the host.

For striated muscle A and B are the intervals $0 < x_1^\ell < 5.5$ torr and $13 \leq x_1^0 < 50$ torr respectively. The interval B correlate with the oxygen concentrantions along the blood capillaries in muscle. (Gonzalez-Fernandez and Atta).

By an appropriate choice of variables the diffusion-reaction equations describing the facilitated transport can be expressed by two first order differential equations and a quadrature. Their numerical solution appears to be more effectively found than by using current methods. Work to incorporate discrete consumption sites (mitochondria) is in progress. (Gonzalez-Fernandez and Kyner).

Auditory Physiology.

A minimal model has been developed which accounts qualitatively for various features of mammalian cochlear processing. The mathematical formulation synthesizes several functional components which include: (i) a linear moodel for the traveling wave of basilar membrane motion; (ii) a linear micromechanics model for coupling the fluid motion to displacement of inner haircell (IHC) celia; (iii) a saturating, nonlinear, instantaneous transduction of displacement to ionic conductance of the IHC; and (iv) a model for electrical response of the IHC which includes the capacities and conductances of IHC apical and basal membranes. Parameter estimates for the model are derived from the experimental literature and responses fall in the physiological range. Our present solution methods yield results applicable to the low frequency range (<2000 Hz). This simplified model shows that the nonlinearity of celia displacement to IHC conductance is adequate to account for various observed nonlinearities in frequency response for increasing stimulus intensity such as broadening of the IHC tuning curve and downward shift of the frequency of peak response. (Shamma, Chadwick, Wilbur and Rinzel).

Speech evoked activity has recently been recorded in a large population of auditory nerve fibers. A major question arose regarding the coding of the speech signal parameters on the nerves and the strategies and processing that the central nervous system might employ to extract these parameters from temporally coded features. Various algorithms have been proposed in the past but no realistic neural networks have been found or conceived to implement them. We have developed an algorithm (as a model neural network) based on a common and a well understood principle of sensory physiology - namely lateral inhibition - to process the data. The successful implementation of the algorithm has highlighted new information bearing elements in the auditory code that have previously been overlooked - specifically that of phase. (Shamma, Sachs and Young).

Inhibitory interactions manifested in the responses of auditory cortical unit responses of the squirrel monkey were examined using single and two-tone stimuli. Four types of responses were discovered. One unit type exhibited profound lateral inhibitory influences and extremely sharp frequency tuning. Another showed sustained inhibitory responses to single tones characteristic of output neurons in other nervous structures. (Shamma and Symmes).

Molecular Biology.

The fact that amino acid replacement requires tolerance to changes at the DNA level suggests that there may be fewer constraints on the codons at replacement sites. A wide class of sequence pairs have been examined and statistical evidence has been found which supports this concept. It is also found that there is less bias in the use of codons at replacement sites which is confirmatory evidence. (Wilbur and Lipman).

Over a period of years researches at the National Biomedical Research Foundation (Georgetown) under the leadership of M. Dayhoff have collected and dated on point accepted mutations in proteins. This led to the PAM matrix model of protein evolution by point mutations. We have analyzed this model and find that it predicts amino acid replacements which require two nucleotide mutations at a rate less than one hundredth of that observed in the data. Part of this discrepancy may be explained by an error in constructing the matrix but a revision in the basic model also seems necessary. (Wilbur).

The relationship of exons to protein function is being studied by examining the dispersion in conservation of the exons within a number of eukaryotic proteins. This method promises to yield more definitive results than previous analyses because of the advantages of an adequately large sample size. (Lipman, Wilbur, and Burks).

We have developed a new algorith for rapid similarity searches of protein data bases. The first stage of the algorithm is similar to that of Wilbur and Lipman (PNAS, 1983) however the modifications made have greatly improved the speed and sensitivity of the algorithm. The algorithm allows sensitive data base searches to be performed on widely available microcomputers. (Lipman and Pearson).

Efforts to inform and educate molecular biologists about the new computational tools for sequence analysis have continued. A short course on computational methods was conducted for the biologists on campus and was attended by more than 100 scientists. (Lipman).

There has been a recent upsurge in interest in cellular automata. To some extent this is inspired by an attempt to understand parallel processing and other innovative computer architectures. However cellular automata are also of interest for their presumed relevance to complex biological systems. We have developed an analytical theory for making statistical predictions about local pattern generation in a class of simple cellular automata. (Wilbur, Lipman and Shamma).

With the increased speed of computers the miltropolir algorithm has led to the development of simulated annealing as a method of optimization. Impressive results have been obtained on complex problems such as a chip design, pattern recognition, and image reconstruction. Little if anything seem to be known about the class of problems where the approach is productive or about optimal annealing schedules. We have undertaken to calculate optimal annealing schedules for some simple examples in order to understand the limitations of the method. (Wilbur).

Renal Physiology.

In earlier studies it was found that the solution manifolds of kidney models have multiple solution branches; research on this problem has continued. The results for a four tube model of the renal medulla include an existence theorem for solutions of the equations describing the model, the derivation of an exact solution for a limiting form of the model equation, and numerical studies of the exact solution. These showed that the solution manifold is folded into three sheets: an upper and lower stable sheet, and a middle unstable sheet. The upper sheet consists of highly concentrating solutions to the model equations, and on this sheet the concentration ratio is determined by the dissipative loss of solute caused by inefficient vascular exchange. With no loss, the upper sheet is not present, and the solution manifold contains at most two sheets. (Garner, Kellogg and Stephenson).

Qualitatively similar results have been obtained for a multinephron, multisolute model of the mammalian kidney. In this study a method of numerically solving the differential equations describing solute and water flow in the model by a combination of Newton and continuation techniques is described. This method is used to generate a connected component of the steady state solution manifold of the model. As in the simple four tube model a three dimensional section of this manifold is shown to be convoluted with upper and lower sheets of stable solutions connected by an unstable middle sheet. Two dimensional sections of this surface are followed from a trivial constant profile of concentrations in the nonconcentrating kidney to the profiles of the maximally concentrating kidney. Study of these sections shows that for a given choice of model parameters there may exist no solution, there may be a unique solution, or there may be multiple solutions. A study of the time dependent solutions shows that the dynamic transition from the lower to the upper state and return may be via a hysteresis loop. (Mejia and Stephenson).

Further studies have been carried out on the effect of various parameter modifications on the concentrating mechanism. These studies have used p rimarily a central core model of the renal medulla. This model utilizes CONKUB, the conversational pathfollower, to follow the effect of the changes. Anatomically the model includes short and long nephrons discharging into a common collecting duct and the renal pelvis. These studies have verified that with an idealized set of parameters it is possible to generate a significant concentration gradient in the inner medulla with no active solute transport out of the thin ascending limb of Henle's loop. When experimental values are substituted for the idealized set, there is substantial flattening of the medullary concentration profiles. This we interpret as being primarily caused by the permeability ratios of the loop of Henle and the collecting duct. This decreases water extraction from the descending limb of the loop of Henle and, as a consequence, salt diffusion out of the thin ascending limb. Appropriate adjustment of the water and urea permeabilities of the inner medullary collecting ducts and of the epithelium separating the renal pelvis from the papilla gives interstitial profiles of salt and urea that are consistent with those experimentally observed. The medullary collecting duct is not, however, completely osmotically equilibrated with the interstitium. These studies have given us much greater insight into the various factors affecting the concentration of urine, but so far we do not have a theoretical model of the concentrating process in the inner medulla that is entirely consistent with the experimental observations. (Kaimal, Mejia and Stephenson).

A single nephron model for the transport of p aminohippurate (PAH) and its analogues through the kidney has been investigated. One of the new features of the model is that it incorporates the epithelial cells surrounding the proximal tubules. These cells are the main anatomical sites of transport of PAH. The kinetics of PAH transport is described by a set of linear conservation equations. A stable numerical scheme that is backward Euler in both space and time coordinates is used to analyze the mathematical problem. We obtain results that are compatible with those of previously published models and with experimental data. An advantage of the present model is that it could be used to evaluate radioisotope images in terms of transport coefficients, flow rates, etc. Thus, these imaging techniques could be used to evaluate renal function in a more meaningful manner. (Kaimal, Kellogg and Stephenson).

CONKUB, a conversational program for tracing solutions of large systems of nonlinear algebraic equations has undergone continued development. Many computations have been performed with it, including solution of renal models and tracing of tortuous turning and bifurcating solutions of problems in the literature. Several tutorials have been prepared to illustrate its use. (Mejia).

The application of symbolic manipulation to simplify or solve models of biophysical phenomena has continued. Radial gradients due to diffusion in permeable flow tubes are being computed where there is production or consumption of species due to chemical reactions. (Mejia, Knepper and Star).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 13,001-11 MRB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Mathematical formulations and analysis relevant to experimental neurophysiology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. Rall	Senior Research Physicist	MRB NIADDK
Others:	I. Segev	Fogarty Fellow	LNC NINCDS
	J. P. Miller	Guest Worker	MRB NIADDK

COOPERATING UNITS *(if any)*
Laboratory of Neural Control, NINCDS
Department of Zoology, University of California, Berkeley
Department of Anatomy, Yale University

LAB/BRANCH
Mathematical Research Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	1.5	.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This research involves creating, exploring and testing mathematical models of dendritic neurons that are relevant to experimental neurophysiology and neuroanatomy. Together these models provide a theory that can account for various sequences of events in the soma and dendritic branches of a single neuron, and for field potentials generated by certain cortical populations of neurons. Computational experiments performed with these models provide theoretical predictions that have been compared with experimental results obtained by colleagues with motoneurons of cat spinal cord, and with the mitral cell and granule cell populations of rabbit olfactory bulb. Resulting interpretations contribute to understanding of dendritic synaptic input and of dendro-dendritic synaptic interations.

Results published in the Biophysical Journal and in the Journal of Neurophysiology are summarized in Chapter 3 of The Handbook of Physiology: The Nervous System, Vol. 1, American Phyiological Soc. (1977) and in a Chapter entitled "Functional Aspects of Neuronal Geometry" in Neurones Without Impulses, SEB Seminar Series, Cambridge Univ. Press (1981).

When an intracellular microelectrode and an electronic control circuit are used to voltage clamp a neuron soma, and then a synapse is activitated at a dendritic branch location, it is important to distinguish the current generated at the synapse from the current that is detected experimentally at the soma by the clamping circuit. The mathematical relation between these currents has been derived explicitly and computations are in progress to explore this relation. Results will be presented and discussed in a Monograph to be published by The American Physiological Society this year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 13,002-12 MRB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mathematical description of substrate transport in capillary-tissue structures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J. M. Gonzalez-Fernandez Research Mathematician MRB NIADDK

COOPERATING UNITS *(if any)*
Laboratory of Biochemistry and Metabolism, NIADDK
Theoretical Biophysics Section, IR, OD, NHLBI

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Mathematical Research Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.45	1.15	.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this work is to develop mathematical models of the blood flow and transcapillary exchanges in capillary networks. An effort is being made to incorporate in the models the histological structure of capillary networks as well as different flow patterns from available experimental information. In this model the extraction of substrates with different chemical kinetics at the tissue site will be described. It is expected that this could be used in experimental situations where the extraction of different substrates are measured simultaneously, thus helping to infer the flow pattern features of the microcirculation. In particular a model of the diffusion-consumption of oxygen in striated muscle containing myoglobin (facilitated diffusion) is being developed and pertinent numerical results examined.

A possible adaptive mechanism for the facilitated transport of oxygen by hemoglobin in nematodes (Ascaris lumbricoides) and myoglobin in vertebrate striated muscle has been investigated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 13,004-10 MRB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mathematical description of cellular neuroelectric signal transmission

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Rinzel	Chief, MRB	MRB, NIADDK
Others:	W. Rall	Senior Research Physicist	MRB, NIADDK
	W. J. Wilbur	Medical Staff Fellow	MRB, NIADDK
	S. A. Shamma	Staff Fellow	MRB, NIADDK
	K. Maginu	Visiting Associate	MRB, NIADDK

COOPERATING UNITS *(if any)*

Department of Biology, University of Pittsburgh
Department of Zoology, University of California, Berkeley
Biomedical Engineering & Instrumentation Branch, DRS

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NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.7	2.4	.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project continues to focus on the formulation, analysis, and biophysical interpretation of mathematical models which describe various aspects of neuroelectric signaling for individual neurons. Among the topics of current interest are: (i) integration of synaptic input delivered to the soma and dendritic branches of a neuron; (ii) propagation of action potentials along axons; (iii) stimulus-response and threshold properties for repetitive-firing of action potentials; (iv) complex bursting patterns of membrane potential oscillations which arise through endogenous membrane properties and/or interneuronal coupling.

Because qualitatively related mathematical or biophysical problems may arise in other contexts, e.g. chemical and biochemical oscillations, or e.g. excitation-secretion coupling, this project may consider models from such applications.

Mathematical models of these phenomena involve systems of linear and nonlinear ordinary differential equations and parabolic partial differential equations. Solutions and their mathematical stability are determined by analytical and numerical methods drawn from both classical and modern applied mathematics. These methods may include finite difference or finite element numerical integration, bifurcation theory, perturbation techniques, and nonlinear dynamical systems theory. One goal of this project is to expose the qualitative mathematical structure for classes of models by exploiting simple, yet physiologically reasonable, equations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 13,014-03 MRB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Probabilistic Analyses of Nucleic Acid Sequences

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: D. J. Lipman Medical Staff Fellow MRB NIADDK

COOPERATING UNITS *(if any)*

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Mathematical Research Branch

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INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 1.04	PROFESSIONAL: 1	OTHER: .04

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has focussed on the analysis of amino acid and nucleic acid sequence data as it pertains to molecular biology and molecular evolution. Continuing areas of interest include:

i) We have provided further theoretical evidence for contextual constraints on eukaryotic coding sequences as well as broadening our understanding of these constraints by quantifying their intensity.

ii) The development of computational tools for molecular biologists, in particular rapid methods of similarity analysis for protein or nucleic acid data bank searches. We have recently modified the protein search algorithm with significant improvements in speed and sensitivity. These modifications allow sensitive protein data base searches to be conducted on widely available microcomputers.

iii) We have developed new, more realistic methods for the estimation of the statistical significance of nucleic acid similarities.

iv) We have initiated a new and different analysis of the relationship of exons to protein function. This research should prove useful in understanding the evolution of exons and introns.

v) Cellular automatons have been used to model a variety of natural phenomena; we have been studying them, in particular, developing methods for predicting their behavior when subjected to noise.

In addition, efforts are continuing on informing molecular biologists of the computational tools available for sequence analysis. A heavily attended short course on computational methods for sequence analysis was provided this year for the molecular biologists on the NIH campus.

PHS 6040 (Rev. 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 13,015-03 MRB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Probabilistic modeling of biological information systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. J. Wilbur	Medical Staff Fellow	MRB NIADDK
Others:	R. Chadwick	Biomedical Engineer	BEI DRS

COOPERATING UNITS *(if any)*

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INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	1.8	.10

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Phenomena related to the nervous system and to biological macromolecules are under study. An analysis of the Stein model of neural integration and firing in the presence of both excitation and inhibition has been completed. A model of cochlear processing of auditory stimuli is being developed. Hair cell transduction is the only nonlinear component in the model and this suffices to explain well known nonlinear auditory characteristics. Cellular automata are under consideration as a possible approach to understanding certain types of neural networks. An analytical procedure for predicting the statistical distribution of local patterns in a class of simple automatons has been developed.

With regard to macromolecules progress has been made in several areas. Context dependent methods of sequence comparison have been investigated and conditions which make such methods metrics on the space of sequences have been found. Refinements in the Monte Carlo simulations used to test the significance of sequence similarities have been made. Relaxed constraints on DNA at amino acid replacements have been discovered. The relative frequencies in the use of synonymous codons in DNA has been analyzed. These frequencies should change under the fixation of mutations unless they are in dynamic balance. Such a dynamic balance has been found for certain classes of sequences and this has led to an estimation of fixation rates in such cases. The PAM matrix model of protein evolution has been analyzed and an error discovered in the construction of the matrix and a need to modify the model demonstrated.

A third area of study deals with the foundations of quantum mechanics. An interpretation has been developed which resolves the measurement paradox and avoids the clash of quantum mechanics, in its usual interpretation, with realist philosophy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 13,016-01 MRB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
Mathematical Analysis of Reaction Diffusion Systems

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI:	K. Maginu	Visiting Associate	MRB NIADDK
Others:	J. Rinzel	Chief, MRB	MRB NIADDK

COOPERATING UNITS (*if any*)

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INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.31	1.3	.01

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

We can observe propagation of excitation waves in some types of spatially distributed excitable media. Well known and important examples of such media are the nerve axon as an electrophysiological pulse transmission line and the Josephson junction strip as a cryo-electronic pulse transmission line. The aim of this project is to make clear, through mathematical analysis and numerical computations, general qualitative features of various types of excitation waves observed in the these media, e.g., solitary waves, periodic wavetrains, center waves, trains of mixed waves, standing waves and chaotic waves.

We studied general geometrical properties of the solitary waves and the periodic wavetrains, and introduced some topological characteristics of these waves. By the use of these characteristics, we succeeded in understanding mechanisms of various bifurcations of the waves (i.e. the transitions from one type of wave to another) and in obtaining conditions for the stability and the instability of the waves.

We introduced a kinematic consideration with respect to mutual interactions of impulse waves, based upon the dispersion relation of periodic wavetrains. By the use of this approximate consideration, we could understand some odd behavior of pulse trains such as the instability of equally spaced wavetrains, the formation of unequally spaced periodic wavetrains and the formation of bunches of densely aggregated pulses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 13,017-01 MRB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Sound Processing in the Auditory System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. A. Shamma	Staff Fellow	MRB NIADDK
Others:	J. Rinzel	Chief, MRB	MRB NIADDK
	R. Chadwick	Biomedical Engineer	BEI DRS
	W. J. Wilbur	Medical Staff Fellow	MRB NIADDK

COOPERATING UNITS *(if any)*

Biomedical Engineering & Instrumentation Branch, DRS

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INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	2.7	.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

These projects have considered the processing of sound at various stages in the auditory system. These studies include:

(1) Theoretical models of the peripheral stages of the system. The computed results agree well with experimental findings and help explain several observed nonlinear phenomena.

(2) The development of realistic algorithms (as model neural networks) to process speech evoked activity on the auditory nerve. The results point to new ways of viewing the auditory nerve code.

(3) Analysis of data from neurophysiological experiments in the squirrel monkey cortex. The data lend strong support to the existance of a lateral inhibitory-type of processing even at higher auditory centers.

CHROMATIN STRUCTURE

We have previously described the precise in vitro phasing of histones when bound to a 255 base pair (bp) segment of sea urchin DNA which contains a 5S ribosomal RNA gene. A number of studies this year have utilized that basic sequence to address questions of higher order chromatin structure and the mechanism of nucleosome phasing, or sequence specific DNA - histone interaction.

We have engineered a series of tandemly repeated, variable length DNA fragments which each contain the phasing signals present in the propositus 255 bp segment. Overall repeat lengths of 172, 180, 190, 198 and 207 bp are available. Initial studies concentrated on repeat numbers of 6,9,12,18,25 and 50 for the 190 repeat length fragment; subsequent investigations have used 12- and 18-mers of the 172 and 207 bp repeats. We have demonstrated that core histones interact in a specific fashion with the tandemly repeated DNA segments. Binding occurs to the same region as was observed for the monomeric fragment in all cases; the phasing signal is sufficiently strong to overcome the tendency for histone octomers associated with random sequence DNA or synthetic polynucleotides to interact cooperatively and form a non-native chromatin structure.

When analyzed by electrophoresis, there are marked differences in the conformation of various length-repeat combinations associated with core histones; these are dependent on both repeat number and repeat length. Within the 190 bp series, 6- and 9-mers migrate like or slower than free DNA. At repeat numbers of 12 and greater, the complex migrates more rapidly than DNA, suggesting a folded conformation. Similarly, using 12-mers, the 172 bp migrates like DNA alone while the 190 and 207 bp repeats fold and migrate more rapidly. Addition of histone H5 to these complexes leads to a retarded mobility and heterogeneous appearance for the 172 and 190 bp repeat length fragments. In contrast, the 207 bp repeat fragment migrates even more rapidly when associated with H5, suggesting a further compaction of the structure. This is of high interest since chicken erythrocytes, the source of H5, have a chromatin repeat length of about 210 bp. We are continuing with spectroscopic and morphologic examination of these complexes. We believe that we have generated a set of fragments which should allow a detailed examination of the structure of higher order chromatin and the effects on same of variations in linker DNA length, types of histones, nonhistone proteins, etc.

In an effort to determine the phasing signals for sequence specific DNA-protein interactions, we have created a set of mutations and asked whether they still form phased nucleosomes. At the site 100 bp from the left border of the core particle, one can delete several bp, insert 2 bp, or invert the 70 bp further to the right without effect on phasing, suggesting that the signals reside to the left of this point. At the site 50 bp from the left of the core particle, deletions of 1,5 and 15 bp abolish phasing while deletions of 7 and 10 bp allow phasing. We conjecture that the major phasing signals reside in the central region of the core particle DNA but that ancillary signals to the left of the 50 bp region must be in the proper spatial relationship (viewed on the surface of the double helix of DNA) to these primary signals to allow phasing to occur.

Enhancer elements have been identified in viral and cellular genes; they are sequences which are required for the active transcription of adjacent genes are active in either orientation in many cases either 5' to 3' of the gene. Many have considered that their role might be in alteration of chromatin structure, perhaps by forming a positioned but unusual nucleosome or perhaps by not forming a nucleosome at all. Studies of the 72 bp repeated enhancer of SV-40 suggested that two overlapping phasing situations might exist. The mutated single 72 bp enhancer forms a phased core particle with core histones. In contrast, the RSV enhancer, while associating with histones to form a folded structure, does not form a phased core particle. Whether these results reflect different modes of action of the two enhancers or are fortuitous and bear no relationship to the mechanism of enhancer function is undecided.

Fundamental questions about the mechanisms of phasing in vivo and concerning transcriptional regulation and the effects of chromatin structure thereon must, eventually, be approached at the level of a unique gene.

We have begun a program to ascertain the composition and structure of single genes in yeast. Methods have been developed by others which allow transformation of yeast. In the system we use, yeast have been transformed with a 1453 base pair (bp) circular DNA which contains the TRP1 gene (allowing selection) and the ARS1 element (allowing autonomous replication). The DNA exists as an amplified episomal element and is packaged with histones into chromatin.

We have characterized the organization of the chromatin by mapping cutting sites for several nucleases using a slightly purified plasmid preparation. Four nucleosomes are present in the gene region; they are not stable during extensive micrococcal nuclease digestion and might dissociate or rearrange during this process. In contrast, on the region of DNA which is neither structural gene nor AR three precisely phased, stable nucleosomes are present. These two regions are separated by two 160 bp regions which do not appear to be packaged with histones into nucleosomes. One region is located near the 5' end of the TRP1 structural gene and one near the 3' end of the gene and the ARS segment. Both these regions are sensitive to digestion by micrococcal nuclease and DNase I. The structure of the regions might relate to transcription initiation and termination or to replication origin function.

Knowlege of the structure of the plasmid allows us to insert or delete DNA segments and address mechanisms of nucleosome phasing and the origin of the nuclease sensitive regions. We have inserted 75, 215 and 155 bp of DNA at a site between two of the three stable nucleosomes. For the first two cases, the three nucleosomes originally resident on the TRPARS circle occur in the identical position, suggesting sequence-directed specificity as the basis for their positioning. For the third, two remain positioned while a third is displaced; in this case, it is possible that a phasing signal on the inserted DNA is stronger than that present in the yeast sequences. Other constructions, currently being analyzed, address the nature of the sensitive region at the 5' end of the gene and will allow distinguishing whether the 3' gene - ARS sensitive region results from replicator function or transcription termination.

In order to investigate the compositional bases for these four chromatin regions on the simple small DNA, isolation of the chromatin in biochemical quantities and pure form is mandatory. Only then can one determine, e.g. whether the unstable nucleosomes contain acetylated histones and the stable ones non-modified, what proteins interact with the two sensitive regions. We have developed methods to isolate nuclei which retain the episomal chromatin, elute it from these nuclei, and fractionate it using sizing columns, isopycnic and velocity centrifugation.

Currently, within six days, the minichromosome can be purified about 27000 fold with an overall yield of about 20%. The final preparation has no discrete nucleic acid other than the plasmid DNA. Nuclease sensitivity and nucleosome positions are similar if not identical to those in the cruder preparations. Selected electron micrographs show a circular element with seven nucleosomes, as predicted by mapping studies above. Protein composition of the minichromosome shows four bands which comigrate with chicken or yeast histones and about 24 bands of higher molecular weight. Twelve of the bands migrate with mobilities which are not inconsistent with the peptides present in purified yeast RNA polymerase II, isolated in this laboratory by Dr. Grant Bittter several years ago. These results, while preliminary, give us hope that we will be able to study in detail the compositional and structural bases for functionally different chromatin regions at the level of an unique gene.

Eventually, we will extend such studies to examination of regulated genes in yeast. We have constructed plasmids which contain the CUP1 (metallothionein-like) and PHO5 (repressible acid phosphatase) genes and will use these to study chromatin structure-function relationships on a given gene in the transcriptionally active and inactive states.

DEVELOPMENT AND DIFFERENTIATION

An attractive system for study of the chromatin structure of developmentally regulated genes is the early histone gene family of sea urchins. This tandemly repeated, 400 copy/genome gene is expressed early after fertilization, directs the synthesis of nearly one-third of mRNA at the 100-200 cell stage, and is rerepressed at the blastula stage. Using now standard methods of mapping nuclease cutting sites, we have studied the chromatin structure of this gene family during the development of Strongylocentrotus purpuratus.

Prior to active transcription of these genes, and, following rerepression of transcription, the chromatin structure of the gene family is similar, with well placed nucleosomes and no sites which are hypersensitive to DNAase I. In contrast, when the genes are active, nucleosomes are infrequent and randomly placed relative to one another. Further, regions hypersensitive to digestion by nucleases appear in the 5'-flanking regions of the genes for histones H2A and H3. The appearance of such sites has been documented by others for genes which are activated during development or differentiation; the disappearance of such sites is nearly unique, suggesting that there may be fundamental differences in the chromatin structure of genes of terminally differentiated cells and cells in early developmental stages.

The sensitive regions actually consist, when mapped at high resolution, of three, equidistantly spaced loci which are digested by the nuclease. The span of the region is 150-160 base pairs. Examination of the DNA sequences of the sensitive regions reveals a sequence similar to the consensus core sequence for viral enhancers at one border of the sensitive region. Further, in one case this sequence is followed by a similar sequence 21 base pairs to the 5' direction and in the case of a second, two similar sequences occur in the succeeding 40 base pa again in the 5' direction. The position of the enhancer-related sequences and sensitive nuclease regions relative to the transcription initiation site differs for the two regions studied, suggesting that the hypersensitive sites are not merely a consequence of the act of RNA initiation, but reflect regulatory protein interactions with DNA. There are similarities of these findings with another developmentally regulated gene sequence, the SGS 4 glue protein locus in Drosophila.

Salt washing experiments suggest that the hypersensitive sites flanking the histone genes are due to interactions of nonhistone proteins with the DNA. Thus, washing nuclei from stages where the genes are active with 0.3-0.4 M NaCl (which removes many nonhistone proteins but does not alter histone-DNA interactions in bulk chromatin) leads to disappearance of the nuclease sensitive region. In contrast, washing nuclei from stages where the gene is inactive with 0.3 M NaCl does not lead to the appearance of sensitive sites. The reiterated nature of the early histone genes should facilitate current attempts to identify a specific regulatory protein for this gene family.

Another gene of interest in early sea urchin development is that coding for collagen. Several lines of indirect evidence involving proline incorporation and enzyme inhibitors have suggested that collagen may be a component of the matrix for spicules; spicules are the skeletal elements of the urchin larva. The spicules are laid down by a limited number of cells whose progenitors are the micromeres, appearing at the 16-cell stage. Micromeres and their progeny are isolatable at all stages of development and have been shown by others to undergo differentiation in culture, making them an attractive cell set for study of lineage specific genes.

We have constructed a genomic lambda library from S. purpuratus using partial MboI fragments of about 20 kbp length cloned in the BamHI site of λJ1. The library consists of about 2.5×10^5 clones. It was screened with a cDNA clone to chicken α2 (Type I) collagen. Several positive clones have been isolated which hybridize to the triple-helical coding region of the chicken cDNA clone and not to highly repetitive genes of sea urchins. A subclone of one of these, after four cycles of purification, has been isolated and is currently being sequenced to ascertain whether we have been able to isolate the gene for a sea urchin collagen.

Dictyostelium discoideum is a simple eukaryote which undergoes a well defined developmental cycle. When starved, vegetative amoebae initiate a wave of cAMP pulses, spaced 6 minutes apart, which lead to aggregation of about 10^5 cells, formation of a migrating slug, and eventually differentiation into a fruiting body with stalk and spore cells. The cloned gene segment called M4 has been under study in this laboratory. M4 contains two genes with different properties. One is active in vegetative cells and becomes repressed at about the time of the cAMP pulsing which leads to aggregation. We have now been able to mimic this repression in shaken culture, where the cells do not differentiate, by exogenous pulsing with low concentrations of cAMP. In the absence of cAMP or with continuous high levels of the nucleotide, the gene is not repressed. Further, cells which are induced to dedifferentiate, by refeeding, rapidly express the gene. In this case, nuclear RNA homologous to the gene appears temporally before cytoplasmic mRNA, suggesting a transcriptional basis for the regulation.

The other gene in the M4 clone is regulated in opposite fashion from the first, expression increasing during development, and is transcribed in a divergent direction from the first. This gene consists of repeat and unique elements; the repeat element $(dAAC)_n \cdot (dGTT)_n$ is upstream from the transcription start site. This element is present in about 100 other locations in the Dictyostelium genome. In several other cases, the sequence is transcribed, asymmetrically, and present in mRNA. The repeat sequence is part of a developmentally regulated family, both unique and repeat sequences having a similar temporal pattern of expression. Certain features of the repeat are "enhancer-like", in its variable position relative to regulated genes, others are not, its constant polarity relative to transcribed sequences. An attractive feature of this gene segment is the presence of all controlling regions for two differently regulated, developmentally modulated genes on the 1.5 kbp of DNA separating the structural genes.

A recent addition to the laboratory and its research program is the group headed by Jurrien Dean which studies pre-implantation embryogenesis in mouse. This group has previously studied the synthesis and post-synthetic modification of three sulfated glycoproteins, ZP1, 2 and 3, which form the zona pellucida, a glycocalyx surrounding the mammalian oocyte. More recently, they have isolated and characterized monoclonal antibodies specific for (a) ZP2, (b) ZP3, or (c) a shared epitope between ZP2 and 3. These have been shown to bind specifically to oocytes when injected into mice and can be utilized for zona-like protein localization using immunoscintigraphy. Passive immunization of mice with these antibodies leads to a reversible block to fertilization; in vitro fertilization is similarly blocked.

Recent studies have centered on efforts to clone the genes for the ZP proteins. mRNA isolated from ovary was shown to direct the synthesis, in vitro, of immunopreciptable proteins with mobilities appropriate for the unmodified zona proteins. A cDNA library from murine ovary was generated using the expression phage gt11. Fifty-five clones which expressed fusion proteins recognized by a polyclonal anti-ZP antiserum were selected from initial screening of about 24 x 10^6 plaques. Fifty-one of these remained positive after tertiary screening. A limited subset of them react with monoclonal antibodies specific for ZP3, suggesting that the whole set represents, in fact, cDNA's for zona proteins. Experiments using hybrid selected or arrested translation with the several clones are in progress.

The three zona proteins have been isolated by preparative gel electrophoresis in amounts sufficient to allow sequence analysis. Initial attempts indicated that the amino termini are blocked, necessitating proteolytic cleavage and sequence analysis of peptides to provide the protein data base allowing definitive identification of the cDNA clones after their sequencing.

A model system for the study of differentiation is afforded by transformed MDCK cells; in contrast to the parental line, the transformed cells do not respond to glucagon. A number of inducers restore responsiveness; interesting, prostaglandins induce effectively, but only in the absence of serum. A putative differentiation inhibitor thus exists in fetal bovine serum. Correlated with the removal of serum allowing inducer function, levels of the transformation protein p21 are also decreased in the absence of serum. The relationship among these several observations remains to be established. EGF and a phorbol ester, TPA, also inhibit induction by prostaglandins.

In order to study the development of hormonal sensitivity in these cells, a means of identifying the glucagon receptor as a protein, not by its activity, would be highly desirable. To that end, MDCK cells or their membranes have bee used as immunogens in the production of a monoclonal antibody library. Several members of the hybridoma library are inhibitory for glucagon binding to MDCK cells, suggesting that they may be recognizing epitopes of the glucagon receptor. During these investigations, we have also defined several hybridomas whose antibody binds to MDCK cells, but not to transformed MDCK cells. These may provide probes for differentiation-related cell surface molecules.

PROTEIN CHEMISTRY AND ENZYMOLOGY

The extracellular ribonuclease of Bacillus amyloliquefaciens, barnase, has been studied in this laboratory for some time as a model protein for active site investigations, folding mechanisms, and protein interactions. Extension of such studies seemed to be most appropriately carried out by in vitro genetic techniques. Initial efforts used synthetic oligonucleotides complementary to the putative gene sequence, based on the known amino acid sequence. These did not succeed.

This year, we have used a phage to transfer a B. subtilis plasmid containing the Tn918 transposon into B. amyloliquefaciens. Insertion of the transposon can be selected for and inactivation of various genes determined. Nearly 1% of the surviving bacteria are auxotrophs and a number of these have been identified. Mutants which fail to produce amylase, the extracellular protease, and barnase have been identified. Each of these produces the other two proteins, suggesting that a secretion mutation is not involved.

Subcloning of the mutant DNA sequence in the barnase-minus strain has le to the identification of DNA fragments which hybridize to the synthetic oligonucleotides used previously for cloning attempts. A fragment of this cloned sequence has been sequenced and shown to contain the C-terminal 23 amino acid coding sequence for barnase, confirming that the barnase gene has been isolated.

Introduction of foreign DNA into B. amyloliquefaciens, done in these studies, had not previously been reported. This opens the door to molecular genetic investigations of this interesting microorganism.

Continuing studies of dihydrofolate reductase (DHFR) have included a comparison of the crystallographic structures of the binary complex of the E. coli enzyme and the chicken liver enzyme (from this laboratory) with methotrexate and trimethoprim by David Matthews' group at UCSD. Both inhibitors are similarly bound to the bacterial enzyme. In contrast, trimethoprim binds in a fashion different from methotrexate to the avian protein. Several interactions which are probably important for inhibition are lacking in the trimethoprim-avian DHFR complex, probably accounting for the 5000-fold greater inhibitory power of trimethoprim against bacterial DHFR vs. animal cell DHFR. The non-active site sulfhydryl residue near the amino terminus of animal DHFR has been implicated in previous studies from this laboratory in enzymatic activity. When this residue is modified, the difference in inhibitory activity of trimethoprim and methotrexate is decreased from 5000 fold to about 100 fold.

HORMONAL MECHANISMS

We reported last year the development of a reproducible method for preparation of fat cells with cAMP contents (assessed by the ratio of basal to maximally stimulated cAMP-dependent protein kinase [A-kinase] activities) equivalent to those existing in situ. Lipolysis varies from nil to maximal over a range of A-kinase activity ratios from 0.05 to 0.3, no matter whether cAMP is increased by lipolytic hormones in the presence of adenylate cyclase inhibitors or decreased by adenylate cyclase inhibitors in an adenosine-free and maximally activating medium.

We have now examined the antilipolytic effects of insulin over a range of cAMP concentrations and A-kinase activity ratios, generated by either stimulating basal cells or inhibiting activated cells. Three phases of insulin activity were found. At A-kinase ratios of less than 0.2 (which produce nearly maximal lipolysis), the insulin anti-lipolytic activity correlated well with insulin-induced decreases in cAMP. From activity ratios of 0.2 to 0.4, lipolysis was decreased in excess of that predicted by the decrease in A-kinase activity due to insulin. Finally, at ratios over 0.5, insulin fails to inhibit lipolysis. There is thus a phase of insulin activity which appears to act in a cAMP independent manner. We speculate that the cAMP-independent event may be activation of a phosphatase that inactivates the hormone sensitive lipase in fat cells.

The IC_{50} values for three processes were different depending on whether cells were stimulated with lipolytic agents (120 pM) or inhibited from maximal A-kinase activity in ligand-free medium (600 pM). Decreases in cellular cAMP, the cAMP-related decrease in lipolysis, and the cAMP-independent decrease in lipolysis were all effected similarly by the route taken to achieve a given A-kinase ratio. It appears that occupancy of cyclase-linked receptors alters the sensitivity to insulin for both cAMP linked and independent processes.

Glucose transport studies in insulin stimulated adipocytes are consistent with the conclusion that adenylate cyclase-linked hormone receptors in fat cells are also associated with the processes whereby insulin alters metabolism in a cAMP-independent manner. Removal of adenosine decreased transport 30% without change in A-kinase ratios. Isoproterenol increased A-kinase activity without effect on transport. Combining these two treatments inhibited transport 70% and increased A-kinase to maximal. Higher concentrations of isoproterenol further inhibited transport and this was antagonized by an adenosine receptor agonist.

The adenylate cyclase system includes two guanine nucleotide regulatory elements, Ns and Ni, which mediate the effects of stimulatory and inhibitory ligand-receptor combinations on the catalytic subunit of the complex. Forskolin rapidly activates cyclase in all animal cells; S49 lymphoma cells are no exception. Somatostatin inhibits, with a half-maximal concentration of 10 nM, by 50% the forskolin-induced accumulation of cAMP in S49 cells. This effect is also rapid.

Pretreatment of S49 cells with a phorbol ester leads to a time-dependent loss of the ability of somatostatin to inhibit the forskolin-induced activation of adenylate cyclase; complete loss required 30 min treatment at 37°C. Extensive washing of cells did not reverse the phorbol ester effect, suggesting a modification of a cellular constituent in irreversible or slowly reversible fashion. Phorbol ester treatment led to modest increases in forskolin-induced cAMP accumulation.

Although ineffective in isolated cells of S49 AC⁻ (a cell line lacking Ns), forskolin appears to stimulate cAMP production in isolated S49 AC⁻ membranes; this stimulation was inhibited by somatostatin. A preliminary observation indicates that the phorbol ester reduced the somatostatin effect with these membranes. Thus, we speculate that the phorbol esters may be acting through the Ni subunit of the cyclase complex. Protein kinase C is known to be activated by phorbol esters; it is possible that this activation leads to modification of Ni. The modified Ni may then be incapable of transducing the stimulus from inhibitory liganded receptors to adenylate cyclase.

Transformation of a variety of cell lines leads to depressed adenylate cyclase activity vs. the parental lines. We have evaluated various features of the cyclase system in an attempt to determine the site at which the transformation-related deficiency resides. Ns appeared to be present in similar amounts in both classes and was functionally competent in both as judged by its ability to confer hormone sensitivity when transferred to S49 AC⁻ cells. Cells which possess an Ni do not express it upon transformation. Alterations in the catalytic component of cyclase also do not appear to be causal in the decreased cyclase activity, based on the results of cell fusion experiments. We currently are investigating protein modification in normal and transformed cell membranes in efffforts to understand the decreased adenylate cyclase activity after transformation.

LIPOPROTEINS AND LIPOPROTEIN LIPASE

Apolipoprotein B (apoB) is a constituent of VLDL and a protein of particular interest in that it appears not to exchange among lipoprotein particles. In the rat, apoB exists as three forms, all synthesized by the liver and one synthesized by small intestine. B48, the small form, was cleared from circulation faster then B95 or B100, the larger forms. We now have shown that B95 and B100 also differ in their rates of clearance from circulation. Further, fractionation of lipoprotein particles on the basis of density leads to concomitant fractionation of the forms of apoB. Thus, the lightest LDL contains an enrichment for B100 while the densest LDL contains six times as much B95 as B100. We suggest that liver may produce three populations of LDL which differ in the form of apoB they contain. The relative amounts of these synthesized is a function of the physiological state of the animal and they differ in their metabolic fate. We are now attempting to prepare monoclonal antibodies that are capable of distinguishing the various apoB forms; these can be used, hopefully, to isolate families of VLDL based on their protein content.

The molecular basis for the heterogeneity of the two major forms of apoA-1 has been determined. Isoforms 3 and 4 were isolated and amino terminal sequence analysis performed with the finding that isoform 4 has a 6 amino acid extension, making it a proapo-I to isoform 3. Some processing of proapoA-I to apoA-I can occur in plasma or blood in vitro. It appears that divalent cations are required for the proteolysis. The observation that small amounts of apoA-I are found in tissues after brief amino acid pulses suggests that some processing occurs in the liver also.

We find that the proportion of apoA-I in the proform is similar for total A-I, isolated from lipoprotein particles obtained by flotation. Thus, it appears that proapoA-I can associate with lipid as readily as the mature form. We thus fail to confirm the suggestion made by others that the proform does not associate normally with lipids.

We have studied lipoprotein metabolism in a mutant mouse, cld/cld, with combined lipase deficiency. These mice develop severe hyperchylomicronemia shortly after birth with plasma triacylglycerol concentrations of up to 20000 mg/dl and die in less than three days. Morphologic obervations showed large irregular shaped chylomicrons packing capillaries in several tissues and subnormal amounts of triacylglycerol in tissues.

No lipoprotein lipase (LPL) activity is detectable in serum of cld/cld mice. We find, however, that the amount of LPL-like protein detected with a chicken anti-bovine LPL antiserum in several tissues is 2-5 times greater than that present in normal mice. ^{35}S-methionine is incorporated into a peptide recognized by the antisera in the mutant mice; the protein has an apparent size on SDS gel electrophoresis like that of bovine LPL. The incorporation into this peptide of radioactive precursor was about twice that observed for normal mice. We find that the LPL activity in acetone-ether powders of heart from cld/cld mice is only about 2% of that found in normal mice. Thus, there appears to be production of normal amounts of a mutant, inactive protein in mice with this genetic lesion.

Using this antiserum we have studied the ultrastructural localization of LPL in normal mice and the LPL-like protein in cld/cld mice. In normals, LPL is found primarily over capillary endothelium, in some cases in association with what appear to be chylomicron remnants. In cardiac myocytes, LPL can be found within sarcoplasmic reticulum and in small vesicles at the periphery of cells. In cld/cld mice, there is no LPL immunoreactive material in association with capillary endothelium. In both heart and brown adipose tissue, there are large vacuoles which contain the LPL-like protein. Thus, the defective protein may also not be transported to capillary epithelium.

We have also studied LPL synthesis and localization in 3T3-L1 adipocytes. Using ^{35}S-methionine and the anti-bovine LPL antisera, we can show that about 50% of newly synthesized LPL is transferred quickly to cell surfaces in the presence of insulin. Active LPL, however, generally accounts for only 10-20% of LPL-like protein in the cells. Rapid turnover of LPL in 3T3-L1 cells is indicated by the short half-life of 30 min for radiolabeled LPL in chase experiments and a similar half-life for LPL-activity in cycloheximide-treated cells. Although LPL is released to the incubation medium, the amounts released are too small to account for the short life of LPL in the cells. Calculations based on the half-life of newly synthesized LPL and the amounts of LPL-like protein recovered in cells and medium indicate that 50-90% of the lipase is degraded in cells.

Evidence for degradation of LPL in 3T3-L1 cells was obtained when ^{125}I-labeled bovine LPL added to the incubation medium was bound, internalized and degraded by the cells. Degradation of bovine LPL was decreased more than 90% when 10 mM NH_4Cl was added, indicating that lysosomal degradation was involved. Evidence that endogenous LPL was similarly degraded was obtained when the amount of LPL-like protein increased 5-fold in cells incubated overnight with NH_4Cl without affecting appreciably the amount in the medium.

Our present findings suggest the following concept for turnover of lipop tein lipase in adipocytes. Synthesis leads directly to catalytically active LP which is either transferred to cell surfaces for release, or to lysosomes for immediate degradation. There is no evidence for precursors which can be activated by hormonal stimuli. Insulin stimulates both synthesis of LPL and its transport to the surface of adipocytes. In vivo, much of the LPL at the surface of adipocytes could be transported to the luminal surface of capillaries, the physiological site of LPL action in tissues. Whether LPL is recycled within adipocytes, or between adipocytes and capillaries, is not known. Staining of fractured cells with antisera and tagged second antibody demonstrated the presence of LPL-like material in rough endoplasmic reticulum and multivesicular bodies as well as on plasma membrane.

Two sets of experiments relevant to the previously presented model for lipid transport by lateral diffusion in a continuum of membranes have been completed. Cultured mouse macrophages accumulate intracellular triacylglycerol when grown in the presence of a lipid emulsion and the absence of albumin. As predicted by our previous studies, lipid particles were continuous with the external leaflet of the plasma membrane. Lamellar structures were present, in some cases extending to intracellular lipid droplets.

Fatty acids are produced by hydrolysis of triacylglycerol if not ionized, they locate primarily in lenses or lipid droplets. Long chain fatty acids are partially ionized at pH 7.8-9.6 to acid-soaps, which locate at lipid-water interfaces and form myelin figures. Fatty acids do not form micelles unless the pH of the aqueous phase exceeds 10. We have studied the effect of pH on visualization and distribution of fatty acids in adipose tissue. Fatty acids were produced by action of hormone-stimulated lipase on intracellular triacyglycerol. Epididyma fat pads of weanling mice were incubated with isoproterenol in glucose-free, a min-free medium at pH 7.4. Omission of glucose reduced reesterification of fatty acids and omission of albumin prevented release of fatty acids to the incubation medium. Tissues were incubated with hormone at pH 7.4 and 38 C for 15 min, fixed with cold glutaraldehyde at pH 7.4, and incubated in solutions at pH 7.4-9.0 and 25° C for 30 min. Myelin figures were visualized, using the freeze fracture technique and electron microscope, in hormone-treated tissue incubated at pH 7.8-9.0, but not in treated tissue incubated at pH 7.4 or in untreated tissues incubated at pH 7.4-9.0. Myelin figures appeared as long multilamellar cylindrical structures, which were located in intracellular channels of adipocytes, extending from the surface of lipid droplets to the extracellular space, and in the extracellular space near adipocytes.

We conclude that myelin figures observed in these tissues are composed of acid-soaps formed by partial ionization of fatty acids produced by intracellular lipolysis. Our inability to visualize fatty acids in treated tissues incubated at pH 7.4 suggests that fatty acids in living tissues may be protonated. This possibility is supported by our recent observation that myelin figures formed from oleic acid at pH 8.1 were transformed to oil droplets when pH of the aqueous phase was reduced to 7.0. Protonated fatty acids in tissues could be expected to locate in lipid droplets, between leaflets of membranes and in leaflets of membranes if surface pressure in the leaflets did not exceed 32-34 dynes/cm.

OTHER ACTIVITIES

Epidemiological studies have suggested that high intake of pro-vitamin A carotenoids may be a preventive factor in carcinogenesis. We have now developed a method which allows the analysis of the carotenoids present in human plasma using HPLC. The method required only 0.1 ml of plasma and 13 minutes for analysis. Using this method we have defined the major plasma carotenoids to be leutin, zeaxanthin, two isomers of cryptoxanthin, lycopene, α- and β-carotene, and an unknown dihydroxycarotene.

The Large Scale Unit has provided bacteria and other cells to many investigators at NIH and elsewhere. Large scale tissue processing was performed as a preliminary step in enzyme purification. Collaborative projects with other NIH groups were carried out, including growth of retinoblastoma cells, calmodulin purification, analysis of hormone-like activities in prokaryotes, isolation of pertussis toxin and preparation of bacterial polysaccharides.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 15004-09 LCDB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Hormone Responsiveness During Cellular Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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COOPERATING UNITS *(if any)*

None

LAB/BRANCH

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SECTION

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TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.2	2.5	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Glucagon responsiveness was selectively lost in a dog kidney cell line, MDCK cells, after transformation by Harvey murine sarcoma virus and this loss can be restored to the transformed cells by culturing the cells in the presence of various inducers, including buytyrate and prostaglandin E-1 or E-2. The induction by prostaglandins is seen only when the treatment is carried out in the absence of serum. This inhibitor of differentiation in the serum has been partially purified. However, the nature of this factor, other than its ability to completely inhibit the induction, remains to be identified. We also found that EGF and a phorbol ester (TPA) inhibit the induction of glucagon sensitivity by PGE-2. The inhibitory effect of TPA is seen at a concentration as low as 0.1 nM. We have observed that acute treatment of normal MDCK cells with TPA leads to loss of hormone response, however, transformed cells appear to be resistant to this uncoupling effect of TPA. Therefore, it is likely that TPA inhibits the induction process itself rather than the expression of induced glucagon sensitivity. TPA is known to activate protein kinase C, which can phosphorylate tyrosine residues in proteins. We are currently studying the involvement of protein kinase C in the susceptibility of normal cells and resistance of transformed cells to the uncoupling effect of TPA. We are also exploring the possible interaction between protein kinase C and the cyclic AMP-dependent protein kinase, since TPA antagonizes the induction by PGE-2, which seems to be mediated by cyclic AMP. We have continued our effort to raise antibodies against glucagon receptors and other cell surface markers which are modulated by cellular differentiation. We have recently, using intact MDCK cells and plasma membranes from rat liver or MDCK cells as antigens, attempted to prepare monoclonal antibodies. Our initial results indicate that we have monoclonal antibodies inhibiting binding of ^{125}I-glucagon to MDCK cells. We are now expanding the hybridoma cultures and the conditioned media will be further characterized to establish the nature of these antibodies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 15005-09 LCDB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Adipocyte Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)*

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Lipolytic modulation in adipocytes by adenylate cyclase effectors is due to changes in cellular cAMP concentration, measured indirectly by assessing the activation state of cAMP-dependent protein kinase (A-kinase). Insulin, by contrast, exhibits both cAMP-related and -independent antilipolytic actions. Moreover, loss of the cAMP-independent insulin effect is related to increased kinase activity, suggesting that this mode of insulin action represents activation of a phosphatase that inactivates the lipase.

The above insulin actions are seen both in cells stimulated by addition of lipolytic hormones and in cells stimulated by removal of adenylate cyclase inhibitor, adenosine in this case. However, hormone-stimulated cells are 5-times more sensitive to insulin than are cells stimulated by adenosine removal, and this phenomenon occurs with both cAMP-related and -independent insulin actions. The key observation is that occupancy of adenylate cyclase-associated receptors modifies insulin action on processes apparently unrelated to cellular cAMP concentrations, a conclusion supported by glucose-transport studies. Although beta adrenergic agonists decrease and adenosine receptor agonists increase insulin-stimulated transport, neither affect appears to be attributable to changes in A-kinase activity.

The above studies answer long-standing questions on the importance of insulin's ability to lower cAMP in inhibiting lipolysis. Under conditions of moderate lipolytic activity insulin acts primarily by decreasing cAMP, but at relatively high lipolytic rates insulin acts via a cAMP-independent mechanism, presumably a phosphatase activation. Perhaps more importantly, the data indicate that adenylate cyclase-linked receptors regulate insulin actions quite independent of any effects they might have on cellular cAMP concentrations, suggesting that these receptors are associated also with molecules other than adenylate cyclase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 15100-14 LCDB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Protein-Nucleic Acid Interactions: Chromatin Structure and Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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None

LAB/BRANCH

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SECTION

Section on Developmental Biochemistry

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.0	7.0	

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have previously described a precisely phased nucleosome core particle generated by in vitro interaction of chicken histones with a 255 bp cloned segment of sea urchin DNA containing a 5 S rRNA gene. We have created a series of mutants of this DNA to examine phasing mechanisms; mutations on the right hand side of the sequence do not affect phasing while those on the left suggest that two distinct regions are involved in histone-DNA interactions. DNA constructions derived from tandem repeats of this sequence and its derivatives are under study as models for higher order chromatin structure. The tandem repeats associate with histone octamers in a manner equally precise to the monomer DNA. Repeats over 12 in length appear to adapt a more compact conformation than those less than that length.

Studies of the TRP1/ARS1 yeast episomal chromatin continue. The plasmid has four distinct types of nucleoprotein interactions. Chimaeric plasmids based on this episome are allowing determination of the features involved in both nucleosome phasing and generation of nuclease sensitive sites. We have isolated the plasmid as chromatin in nearly pure form and are now able to investigate both the structural properties of unique yeast genes and the protein components involved in generation of those structures.

Studies of the developmentally regulated early histone gene set of sea urchins are completed. Three histone genes are preceeded by nuclease hypersensitive regions; each region consists of a common set of sites, equidistantly spaced and spanning 150 bp of DNA. A number of investigations suggest that these sites are created by proteins interacting with enhancer-like DNA sequences and may be involved in control of genetic activity of this early embryonically expressed gene family.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 15102-24 LCDB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of a Ribonuclease and its Inhibitor from Bacillus amyloliquefaciens

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Robert W. Hartley	Research Physicist	LCDB	NIADDK
Other:	Christopher Paddon	Visiting Fellow	LCDB	NIADDK

COOPERATING UNITS (if any)

C. Chothia, MRC Laboratory of Molecular Biology, Cambridge; G.G. Dodson, Dept. of Chem., University of York; Y. Mauguen, Centre Pharmaceutrique, Univ. Paris-Sud

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Developmental Biochemistry Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Two proteins, barnase, the extracellular ribonuclease of Bacillus amyloliquefaciens, and barstar, its intracellular inhibitor, are used as a model system for the study of protein folding and protein-protein interactions. Barnase is one of an homologous group of ribonucleases occurring in both prokaryotes and eukaryotes. Amino acid sequence determination and X-ray structure characterization are being carried out along with theromydynamic studies of folding reactions. A peptide complementation system for barnase has been developed which will be used to investigate the roles of specific residues in both folding and enzymatic activity.

Recombinant DNA techniques are being applied to the project with three major aims: 1) to facilitate production, 2) to examine the structural and control sequences of the genes and 3) to tailor specifically designed modifications in the sequences to test theories of protein folding.

A technique of transposon mutagenesis has been adapted to B. amyloliquefaciens. This permits the identification and cloning of any gene for which there is a functional test. A gene controlling the synthesis of barnase has been found and, with its inserted transposon, cloned. This DNA has been partially sequenced and identified us the barnase structural gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 15200-24 LCDB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Folic Acid and Vitamin A

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Bernard T. Kaufman Research Chemist LCDB NIADDK

COOPERATING UNITS (if any)

Dr. John G. Bieri, Scientist Emeritus, NIADDK
Dr. David Matthews, University of California, San Diego

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Nutritional Biochemistry Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.3	1.3	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Dihydrofolate reductase is the site of action of several important drugs (i.e., methotrexate, trimethoprim) widely used int he chemotherapeutic treatment of numerous malignancies, certain bacterial infections, psoriasis, arthritis and other human diseases. Understanding the detailed mechanism of action of this enzyme and its inhibition by these drugs as well as its unique molecular biology should facilitate a rational approach to drug design and chemotherapy. Thus we continue our studies on the isolation and characterization of hepatic dihydrofolate reductase from various animals with the result that it was possible to compare the interaction of trimethoprim and methotrexate with the reductases from animals (chicken liver) and bacteria (E. coli). The correlation of binding and enzymatic inhibition with the 3-dimensional structure of the enzymes resulted in an explanation of the unique antibiotic properties of trimethoprim as compared with methotrexate.

The development of a rapid HPLC procedure for carotinoids showed that eight major compounds are present in plasma: leutin, zeaxanthin, an unknown dihydroxy carotene, two isomers of cryptoxanthin, lycopene, α-carotene and β-carotene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 15302-14 LCDB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Biochemical Studies of Hepatic and Intestinal Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Dr. H.G. Windmueller	Research Chemist	LCDB	NIADDK
Others:	Dr. Mary B. Sliwkowski	Staff Fellow	LCDB	NIADDK
	Mr. Albert Spaeth	Chemist	LCDB	NIADDK

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Nutritional Biochemistry Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are continuing to use the rat as a model to study the biosynthesis and metabolism of plasma apolipoprotein B (apoB), the structural protein of very low density lipoproteins (VLDL), and apoA-I, the major protein of high density lipoproteins (HDL). Evidence has been obtained that rat livers produce three forms of apoB, differing in their apparent molecular weights. Furthermore, the relative amount of the three forms produced appears to be regulated by diet and perhaps other physiological variables. This seems important because in other studies we have shown that all three forms of apoB are independently metabolized, the relative circulating lifetimes being as follows: B-95 > B-100 > B-48. Together, these findings point to a complex mechanism in the rat whereby the metabolic fate of hepatic VLDL and its lipid component is physiologically regulated by the form of apoB it contains.

Amino-terminal amino acid analysis of the two major apoA-I isoforms from rat plasma revealed that the more acidic isoform is a proform containing a 6-amino acid extension at the NH_2-terminus. Similarly, the more basic isoform was identified as mature apoA-I. Both rat liver and small intestine synthesize and secrete _in vivo_ predominantly proapoA-I, which is slowly processed in the circulation to mature apoA-I (∿80% complete in 10 h). Slow and incomplete processing (∿20% complete in 10h) occurs in whole, heparinized blood or in plasma incubated at 37°C _in vitro_. Thus, while the protease presumably required for the conversion is present in plasma, it may be labile. The protease also exhibits an apparent requirement for divalent metal. Additionally, circulating rat proapoA-I, like mature apoA-I, is virtually all associated with lipoproteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AM-15400-10 LCDB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Hormones, Lipoprotein Lipase and Lipid Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Robert O. Scow, Chief, Endocrinology Section, LCDB, NIADDK

Others: Sidney S. Chernick, Scientist Director, LCDB, NIADDK
Mary M. Garrison, Physiologist, LCDB, NIADDK

COOPERATING UNITS *(if any)*

Division of Atherosclerosis and Metabolism, Mt. Sinai School of Medicine,
New York, NY 10029

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Endocrinology Section

INSTITUTE AND LOCATION

NIH, NIADDK, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
3.3	1.8	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We studied synthesis, secretion and degradation of lipoprotein lipase (LPL) in cultured murine 3T3-L1 adipocytes using antiserum to bovine milk LPL raised in chickens. The antiserum proved effective for radioimmunoassay, immunoprecipitation and immunoinactivation of mouse LPL. The major radioactive protein precipitated by bovine-LPL antiserum from cells incubated with S-35 methionine had the same mobility on SDS-PAGE as bovine LPL (Mr = 47,000). Studies with S-35 methionine showed that newly synthesized LPL is catalytically active, and up to 50% is transferred immediately to cell surfaces in the presence of insulin. Active LPL accounted for less than 20% of LPL-like protein in cells. Rapid turnover of LPL was indicated by a half-life of 30 min for radiolabeled LPL in chase experiments and LPL-activity in cycloheximide-treated cells. Calculations showed that more than half of the enzyme was degraded in cells.

A single recessive mutation, cld, on mouse chromosome 17 causes a deficiency of LPL activity. Mice homozygous for this defect develop lethal hyperchylomicronemia within 2 days after birth with plasma triacylglycerol conc as high as 20,000 mg/dl. Radioimmunoassays, using bovine-LPL antiserum, showed that the amounts of LPL-like protein in diaphragm muscle, heart and brown adipose tissue of cld/cld mice were 2-5 times larger than that in unaffected mice. In vivo incorporation of S-35 methionine into LPL-like protein in 40 min was normal or above normal in diaphragm muscle, heart, brown adipose tissue and liver of cld/cld mice. The major radioactive protein precipitated by bovine antiserum from all tissues in both groups of mice had the same mobility on SDS-PAGE as bovine LPL. Our findings show that tissues in cld/cld mice can synthesize normal amounts of LPL-like protein, but the protein has little catalytic activity. Turnover of LPL-like protein may also be impaired in tissues of cld/cld mice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AM-15401-12 LCDB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Transport of Lipids, Hormones and Enzymes in Tissues, Cells and Membranes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)*

PI: Robert O. Scow, Chief, Endocrinology Section, LCDB, NIADDK

Others: E. Joan Blanchette-Mackie, Research Biologist, LCDB, NIADDK
Lynn Amende, Staff Fellow, LCDB, NIADDK
Nancy K. Dwyer, Biologist, LCDB, NIADDK

COOPERATING UNITS *(if any)*

Division of Atherosclerosis and Metabolism, Mt. Sinai School of Medicine,
New York, NY 10029

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Endocrinology

INSTITUTE AND LOCATION

NIH, NIADDK, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.3	2.5	2.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We studied with the electron microscope distribution of lipoprotein lipase (LPL) in heart and brown adipose tissue of new born normal and lipoprotein lipase-deficient (cld/cld) mice. Bovine-LPL antiserum raised in chickens was used as the primary immunoreactant and rabbit anti-chicken IgG labelled with ferritin or gold particles as the secondary antibody. LPL was localized over capillary endothelium in normal heart and brown adipose tissue. Label was also found over endothelial projections and along the luminal surface of capillaries. Ferritin label was found in myocytes of normal heart in packets within intracellular channels near mitochondria and in small vesicles near the surface of cells. Very little label was found at the basal surface of endothelium or myocytes. Brown adipose tissue and heart of cld/cld mice contain 3-5 X normal amounts of inactive LPL-like protein. Ferritin label was found over large peripheral vacuoles in both adipocytes and myocytes of cld/cld mice. This finding may reflect impaired transport of LPL to capillary endothelium.

Electron microscopic studies showed that capillaries in heart, brown adipose tissue and heart of cld/cld mice were filled with large irregularly shaped chylomicrons, and parenchymal cells in these tissues contained subnormal amounts of triacylglycerol, reflecting absence of LPL activity in capillaries.

Effect of pH on visualization of fatty acids as myelin figures was studied in adipose tissue undergoing hormone-stimulated lipolysis. Myelin figures were seen, using the freeze fracture technique, only in tissues incubated at pH 7.8-9.0.

Lipoprotein lipase activity was demonstrated at the surface of cultured mouse macrophages (J774.1) by the presence of surface-attached remnants of triacylglycerol-rich particles in incubated-unfixed cells, and development of myelin figures at sites of surface-attached particles in incubated glutaraldehyde-fixed cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AM-15402-09 LCDB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Lingual (Pharyngeal) Lipase and Digestion of Dietary Fat

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Robert O. Scow, Chief, Endocrinology Section, LCDB, NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Endocrinology Section

INSTITUTE AND LOCATION

National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has become temporarily inactive.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 15500-24 LCDB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Large-Scale Processing of Biological Materials

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Joseph Shiloach,	Head, Pilot Plant	LCDB	NIADDK
Others:	Chaim Rubinovitz	Visiting Fellow	LCDB	NIADDK
	David Rogerson	Expert	LCDB	NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Large Scale Unit provides bacterial and eukaryotic cell growth facilities and large scale tissue processing and purification facilities for collaborative studies with NIH investigators. In the past year, 194 requests have been handled. Fermentations of various bacteria, unicellular eukaryotes and tissue culture cells have been performed. Tissues have been processed and initial large scale level enzyme purifications made for about a dozen investigators. Collaborative projects have been carried out with several groups at NIH involving growth of retinoblastoma cells, purification of calmodulin, identification of peptide hormones and prokaryotes and simple eukaryotes, isolation and pertussis toxin and preparation of bacterial polysaccharides.

PROJECT NUMBER

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AM 15502-03 LCDB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Relationship Between Differentiation and Transformation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Suzanne K. Beckner Senior Staff Fellow LCDB NIADDK

COOPERATING UNITS (if any)
Norman Salem, Senior Staff Fellow, LCS/NIAA/ADAMHA
Thomas Y. Shih, Research Scientist, C/FCRF

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Section on Membrane Regulation

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.8	0.5	0.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The adenylate cyclase responsiveness of a variety of transformed cell lines was found to be depressed compared to their parental counterparts, an effect which correlated with the presence of the gene for the viral transforming protein. The guanine nucleotide regulatory protein which mediates stimulation of adenylate cyclase (Ns) was present in identical quantities is both normal and transformed cells, as determined by cholera toxin labeling. Additionally, the ability of Ns from normal and transformed cells to reconstitute hormone sensitivity of unresponsive S49 cyc- cells was identical ruling out a defect in this component as an explanation for the observed results. Several normal and transformed lines were screened for the presence of the guanine nucleotide regulatory protein which mediates inhibition of adenylate cyclase (Ni). Although normal rat kidney cells (NRK) do have Ni there was no difference in the Ni activity in normal and transformed NRK cells. Furthermore, Madin Darby canine kidney cells (MDCK) do not exhibit functional Ni activity, ruling out differential inhibitory input to adenylate cyclase activity as an explanation for depressed adenylate cyclase activity observed in transformed cells. Fusion experiments suggest that the decreased responsiveness of transformed cells does not reflect a defect in the catalytic component of adenylate cyclase since depressed activity is observed when endogenous catalytic activity is inactivated in fused hybrids. An explanation for depressed adenylate cyclase responsiveness of transformed cells is being investigated by comparison of regulation of endogenous protein phosphorylation of normal and transformed cell membranes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 15503-03 LCDB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Developmental Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)*

P.I.:	Alan R. Kimmel	Special Expert	LCDB	NIADDK
Other:	Barbara Carlisle	Chemist	LCDB	NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Developmental Biochemistry Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The M4 region of the Dictyostelium genome contains two divergent transcription units; the 5'-ends of these genes are separated by 1.5 kb. These genes exhibit completely opposite modes of developmental regulation. M4-1 is expressed in vegetative cells but not in cells late in development. Its expression is repressed by developmental pulses of cAMP but is rapidly induced during dedifferentiation. Comparisons of nuclear and cytoplasmic RNA levels during development and dedifferentiation suggest that changes in expression of M4-1 are mediated at the level of transcription. In contrast, the expression of M4-4 increases during development. M4-4 contains a short, developmentally regulated repeat. The increase in expression of the repeated sequence during development parallels that of the unique portion of the M4-4 gene, suggesting coordinate regulation of the M4-4 repeat gene set.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 15504-02 LCDB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Insulin Action and the Regulation of Glucose Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)*

P.I.:	Richard Horuk	Visiting Fellow	LCDB	NIADDK

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Cellular and Developmental Biology

SECTION
Membrane Regulation Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.0	1.0	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Project terminated

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 15505-01 LCDB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Role of GTP-Regulatory Proteins in Regulation of Membrane Processes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Martin Rodbell Section Chief LCDB NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Membrane Regulation Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have shown in previous studies that two types of GTP-regulatory proteins, designated Ns and Ni, are ubiquitously present in the surface membrane of animal cells. A prominent role of Ns and Ni is to stimulate and inhibit adenylate cyclase, respectively. A large number of hormone or neurotransmitter receptors are coupled to these proteins; when occupied by agonists, the receptors promote "activation" of the N proteins with resultant enhancement of either inhibition of adenylate cyclase activity either in cells or isolated plasma membrane preparations.

Recently, I have found that treatment of S49 lymphoma cells with phorbol esters (potent tumor promoting substances) blocks the ability of somatostatin to regulate the inhibition of adenylate cyclase through its receptor coupled to an Ni unit. This response to phorbol esters is prompt (within 5 min.) and very sensitive (half-maximal dose, 1 nM). Concomitant with the loss of somatostatin action was a modest increase in cyclic AMP production in the absence of added stimuli. These findings are consistent with modification of the Ni unit with the result that it is not activated by the liganded somatostatin receptor and is ineffective as an inhibitor of adenylate cyclase. The importance of the finding is that it suggests means of regulating cyclic AMP production other than through receptors that are directly coupled to GTP-regulatory proteins. Protein kinase C, known to be activated by phorbol esters in the plasma membrane, may be responsible for modifying Ni in S49 cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 15506-01 LCDB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Early Mammalian Development: Expression and Function of the Zona Pellucida

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Jurrien Dean	Senior Investigator	LCDB	NIADDK
Others:	Maurice Ringuette	Visiting Fellow	LCDB	NIADDK
	Steven Chamow	Staff Fellow	LCDB	NIADDK
	Donna Sobieski	Chemist	LCDB	NIADDK

COOPERATING UNITS (if any)

Nuclear Medicine Department, CC (Drs. A. Keenan and S. Larson)
Pregnancy Research Branch, NICHD (Drs. D.R. Mattison and B.J. Gulyas)

LAB/BRANCH

Laboratory of Cellular and Developmental Biology

SECTION

Developmental Biochemistry Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.8	2.2	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Pre-implantation development in mammals involves the modulation of gene expression during cell differentiation. The sulfated glycoproteins of the murine zona pellucida consititute a gene family that is germ-line specific and active only during meiosis where its coordinate regulation occurs in the absence of cell division. During oogenesis, the zona pellucida appears as a discrete extra-cellular structure that surrounds the oocyte and contains three glycoproteins designated ZP-1, ZP-2 and ZP-3. We have developed and characterized a battery of rat monoclonal antibodies which are specific probes to the zona proteins. Five bind exclusively to ZP-2, three bind both to ZP-2 and ZP-3 and an additional two cell lines are specific for ZP-3. No mouse tissue other than the ovarian zonae pellucidae are immunoreactive with these antibodies and the zona proteins are antigenically distinct from other previously described extra-cellular matrix proteins. Although ZP-3 is thought to be the species-specific sperm receptor, monoclonal antibodies to either ZP-3 or to ZP-2 appear equally effective in inhibiting in vivo and in vitro fertilization. Parenterally administered antibody localizes to the zonae surrounding growing oocytes in the ovary. After ovulation, the bound antibody prevents the formation of the 2-cell embryo. This effect is reversible after 80 days and the resumption of fertility is associated with the disappearance of anti-zona antibody from the ovary. The presence of the antibodies does not perturb cleavage, the formation of the blastocyst or the hatching reaction. Using immunological probes we have recently screened a λgt11 cDNA library made from polyA enriched RNA of murine ovaries. We have identified 51 positive clones and are in the process of characterizing them. These clones will be used in initial studies to determine the genomic organization of the zona genes and identify elements that may control their coordinate expression.

ANNUAL REPORT OF THE
LABORATORY OF BIOCHEMISTRY AND METABOLISM
NATIONAL INSTITUTE OF ARTHRITIS, DIABETES & DIGESTIVE & KIDNEY DISEASES

The Laboratory has been conducting research in highly diverse areas that include such apparently distantly related fields as endocytosis, differentiation, hemochromatosis, detoxication and the folding of proteins. The element common to each of the subjects summarized here is appropriate to the Laboratories designation; biochemical and metabolic approaches are being brought to bear on major problems encompassed by the Institute's change.

During the year, the Chief of the Laboratory during the period 1970-1984, was awarded the Merck Award by the American Society of Biological Chemists.

A. Mechanism of Receptor-Mediated Endocytosis in Hepatocytes

For those ligands destined to be catabolized, receptor-mediated binding and endocytosis initiates a continuous process which originates at the plasma membrane of the cell and terminates within the lysosomes. To provide a more detailed rationalization of the resulting continuum, selective perturbants have been used to interrupt the flux of ligand through the cell at discrete, operationally-defined stages.

The experimental system used for these studies requires that ^{125}I-asialo-orosomucoid be added to the culture medium of rat hepatocytes in short-term monolayer culture at 4°C, binding to the plasma membrane of the cell in a saturable and calcium-dependent manner. Under these conditions, internalization is negligible. Upon raising the temperature to 37°C, ligand enters the cell rapidly in a relatively synchronous wave. After 30 min, degradation products appear in the culture medium as evidenced by the recovery of acid-soluble radioactivity. A solubilization-precipitation procedure is used to monitor the rate of ligand dissociation from the internalized receptor-ligand complex; the method has been extended to provide a biochemical assay for the segregation step mandatory for concomitant lysosomal degradation of the ligand and preservation of the recycled receptor.

Assays are now available for each step at which the sequence of endocytic events has been successfully impeded by inhibitory agents. Currently, six such loci have been identified: A) The binding of ligand to cell surface receptors or B) The internalization of receptor-ligand complexes is assayed as resistance to displacement by N-acetylgalactosamine. C) The intracellular dissociation of ligand is assayed by the solubilization-precipitation technique. D) The segregation of receptor and ligand is assayed by monensin-mediated reassociation and is inhibited by colchicine plus cytochalasin B. E) The delivery of ligand to lysosomes is assayed by Percoll density gradients and is interrupted by sodium deprivation. F) The intralysosomal proteolysis of ligand is assayed by acid solubility and is prevented by the bacterial tripeptide, leupeptin.

The criteria employed to determine those events either proximal or distal to the locus of inhibition, were examined and have been interpreted in terms of a continuing flux of unhindered ligand through the endocytic pathway.

B. Receptor Regulation and Iron Metabolism

An attempt is being made to define the specific biochemical and biophysical signals that determine the fate of receptors within the cell. Two systems are being studied: the transferrin receptor, using a human erythroleukemia cell line, and the T cell growth factor receptor, using several human T cell lines.

The transferrin (Tf) receptor is a highly regulated molecule. It enters the cell through the classic pathway of endocytosis but only when activated by the appropriate signals. These can be given by ligand by anti-receptor antibody or by activation of protein kinase C using phorbol esters. The last type of activation results in a dramatic increase in receptor phosphorylation. Once inside the cell, ligand-driven receptors recycle to the plasma membrane. In contrast, antibody-driven receptor moves to the lysosomes where it is degraded.

Using normal peripheral blood human T cells, it has been demonstrated that only an agonistic ligand such as IL-2 will stimulate the internalization of the T cell growth factor receptor. Anti-receptor antibodies with antagonistic activity are not internalized.

The regulation of the human Tf receptor by iron has now been shown to occur via alterations in the level of mRNA for the receptor. The cells sense a very labile pool of iron and even the process of down regulating receptors results in a rapid enhancement of receptor biosynthesis. This may explain why all growing cells need to be continuously exposed to Tf. Consistent with the rapid sensing of iron by the genome, is the finding that a small percentage of iron released in the cell by Tf is rapidly delivered to the nucleus.

C. B Cell Proliferation: Mechanism of Triggering Activation and Fate of Receptors

The purpose of this project is to study the mechanism of triggering proliferation and differentiation of those cells involved in the immune response.

Splenic B cells are induced to proliferate upon culture with antibody having specificity for surface membrane immunoglobulins. Cells treated with pronase, washed and then cultured with antibody, exhibited a greater than 5-fold enhancement of DNA synthesis whereas pronase treatment as such was not mitogenic. Cells from immature, mice which essentially do not show a proliferative response to antibody, become responsive subsequent to pronase treatment. In addition, splenocytes derived from strain CBA/N (x-chromosome linked immunodeficient mice) do not synthesize DNA in response to anti-mouse IgM although such cells respond readily to anti-IgM covalently linked to Sepharose. However, after pronase treatment, CBA/N splenocytes responded to free anti-IgM at 40 to 100% of the DNA synthetic capacity detected with Sepharose linked anti-IgM. Murine B lymphocytes in the presence of anti-IgM begin to synthesize DNA at about the 36th hour of culture although the onset of synthesis in response to other B cell reactive mitogens occurs at approximately 18 hours. By contrast, the onset of DNA synthesis by pronase treated cells in response to anti-IgM required only 18 hours. As judged from a variety of experiments, pronase functions by attacking surface sites on B cells as a consequence of its proteolytic properties. Taken together, the findings suggest the interpretation that a pronase sensitive site on the surface of B cells functions to regulate the proliferative response resulting from perturbation of the surface immunoglobulin receptors.

Enhanced phosphorylation of protein in membranes of cells cultured with anti-IgM linked to Sepharose was observed initially at about 16 hours of cell culture and continued thru the 48th hour yielding the same time course as for 3H-thymidine incorporation. However, the bulk of the enlargement of cells (blast formation) occurred prior to 16 hours of culture i.e., before detection of enhanced phosphorylation or DNA synthesis. These findings suggest the possibility that membrane protein phosphorylation may play a crucial role during the committment for entry of cells into S phase.

D. Development of Mammary Gland

Two groups within the laboratory are investigating the problem of growth and differentiation of mammary gland as influenced by hormones, by distinctly different approaches.

1. Hormone-Dependent Development of Mammary Gland

This laboratory had previously reported that insulin is essential for the the accumulation of casein mRNA in explants of murine mammary tissue. Since the rate of accumulation of mRNA is a function of both the rate of its transcription and of its half-life, it was of interest to determine which component is influenced by insulin. Others had shown that prolactin, another hormone essential for the accumulation of murine casein mRNA, markedly increases the half-life of the mRNA and increases its transcription rate in the presence of insulin. Studies here confirm the marked effect of prolactin on the half-life of the mRNA, and demonstrate that insulin is not required for this effect. However, we have also observed that prolactin does not stimulate the transcription of the 25K mRNA gene in the absence of insulin. The mammary system may be the first known instance in which insulin has been demonstrated to exert an essential, positive influence on transcription.

Estrogen-depletion had been shown to render mouse mammary cells incapable of casein gene expression in the presence of insulin, glucocorticoid and prolactin. These cells retain responsiveness to insulin and glucocorticoid, but lack responsiveness to prolactin. The inability to express casein genes was ascribed to a post-binding, prolactin-related lesion. However, recent results with mammary cells from estrogen-depleted rats cast a different interpretation on the role of estrogen in mammary differentiation. The estrogen-depleted rat cells have lost about 90% of their capacity for casein gene expression. They, too, retain full general insulin - and glucocorticoid - responsiveness. However, unlike the corresponding mouse cells, the estrogen-depleted rat cells also retain general responsiveness to prolactin. In the absence of the female sex steroid, rat mammary celsl appear to lose only their unique responsiveness to the lactogenic hormones.

2. Regulation of the Hormone-dependent Growth and Differentiation of Mammary Gland.

Using a primary mammary epithelial cell culture system, epidermal growth factor (EGF), a peptide hormone synthesized and secreted by the salivary gland, stimulated mammary cells proliferation and inhibited mammary cell differentiation. These effects of EGF were apparent at physiological concentrations.

The mRNA sequence of whey acidic protein, a major mouse milk protein, was cloned in full length. The sequence analysis of the cDNA clones revealed that the mRNA contains a 28 nucleotide-long 5'-noncoding region. Three different portions of this region were identified as possible sites of interaction with the 3'-end of 18S rRNA that may facilitate efficient rate of translation of the mRNA.

Cortisol 21-mesylate was previously shown to exert an anti-glucocorticoid action in rat hepatoma cell culture. When this agent was examined in organ culture of mouse mammary gland, it produced a glucocorticoid-like effect in stimulating milk protein synthesis. These results indicate that the effect of cortisol mesylate can be agonist-like or antagonist-like, depending on its target tissues.

The differentiative potential of the lactating mouse mammary epithelial cells were examined in organ culture. The data indicate that in the presence of insulin, cortisol and prolactin, mammary epithelial cells in culture first lose and then regain the ability of synthesizing milk protein without requiring the formation of new daughter cells.

E. Polysaccharides in Morphogenesis

A study on the relative distribution of mannoprotein and glucan in the cell wall of *Saccharomyces cerevisiae* has shown that the mannoprotein forms an external layer that determines cell wall porosity. The linkage between the different components of the cell wall will be the subject of future studies.

The spatial controls that determine localization of the primary septum in yeast is being investigated with the help of a mutant that lays down septa at abnormal locations.

Chitin synthetase from *S. cerevisiae* has been highly purified by a novel procedure. Efforts are being made to obtain antibodies against the enzyme so as to study its cellular localization as well as the relationship between chitin synthetase zymogen and the active form. Experiments are also under way to clone the synthetase gene.

(1 3)Glucan synthetase, the enzyme that catalyzes the synthesis of the major structural polysaccharide in the *Saccharomyces* cell wall, has been studied in several fungi. The activation of the enzyme by nucleoside triphosphates was found to be conserved in species that are only distant taxonomically. The kinetics of inhibition of the synthetase by the antibiotic, papulacandin, suggest that glucan synthetase exists in at least two different forms.

F. Function of Bacterial Outer Membrane Components

A class of *Escherichia coli* outer membrane proteins called porins have been shown to have a role in the expression of K1 capsule biosynthesis. The rate of capsule expression is markedly reduced in porin-negative strains. Collaborative work with Dr. R. Silver (BB/DBP) has demonstrated that newly synthesized K1 capsule is to be found only at the ends of an *E. coli* cell. This cell line lacks the capsule-associate porin protein K.

In collaboration with Dr. L. Leive (NIADDK/LBP), it has been shown that the acrA mutation in E. coli results in hypersensitivity to a variety of antibiotics. The acrA gene has been cloned and the gene product identified as a component of the cytoplasm with a molecular weight of about 30,000.

The "extracellular" DNAse gene of Vibrio cholerae has been cloned and the gene product identified. Although the DNAse is supposed to be an extracellular enzyme, we find more than 90% of the activity associated with the cell.

H. Studies on Lysosomal Cystine Transport

The enzymic hydrolysis of proteins within lysosomes of animal cells produces free amino acids which normally transit the lysosomal membrane to the exterior milieu at a rapid rate. In studies conducted over the past several years we have obtained evidence that transport of the amino acid cystine from lysosomes of normal human polymorphonuclear leucocytes occurs by way of a stereospecific, carrier-mediated process exhibiting saturation kinetics. No exodus of cystine can be detected from corresponding lysosomes isolated from patients afflicted with the inherited childhood disorder cystinosis, a potentially fatal disease characterized by excessive and specific storage of cystine, within the lysosomes of many tissues. The maximum velocity of cystine egress from lysosomes of obligate heterozygotes for cystinosis was found to be one-half that of normal, indicating that clinically unaffected carriers possess half the normal complement of the putative cystine carrier. These observations constitute presumptive evidence for a defective lysosomal cystine carrier as the underlying cause of human cystinosis.

More recent studies have been directed to the further characterization of the lysosomal cystine carrier system in collaboration with W. A. Gahl, NICHHD. It has been found that cystine egress from normal polymorphonuclear leucocytes is stimulated by ATP in the presence of potassium ions. This stimulation is not affected by agents known to block the ATP-dependent lysosomal proton pump (e.g., N-ethylmaleimide, ammonium ions or the protonophore CCCP) and thus appears to be due to a mechanism other than acidification per se, possibly the result of phosphorylation of the cystine carrier.

I. Organelle Biology - The Structure, Function and Biogenesis of the Golgi

Monoclonal antibodies have been raised against proteins of the Golgi apparatus. These are directed towards determinants present on integral membrane protein or peripheral and context proteins. Immunofluorescence microscopy has been used to characterize the morphologic distribution of the antigens recognized by each clone. Several distinct patterns of distribution have been seen. The specific antigen recognized of each monoclonal antibody is being determined by either immunoprecipitation of labelled proteins or immunoblotting. Several clones produce antibodies that recognize the carbohydrate moiety of Golgi glycoproteins. The topological localization with respect to the membrane of th epitopes recognized by the antibody are being determined. This library of monoclonal anti-Golgi antibodies will allow the study of biosynthesis, degradation, dynamics and function of this critical organelle.

J. Enzymatic Basis of Detoxication

A group of about thirty enzymes are ubiguitously distributed among the animal kingdom with the apparent "purpose" of detoxifying foreign compounds. This laboratory has identified and characterized a number of these enzymes.

Recent work has been aimed at conjugation reactions of amines. Specifically, a number of enzymes are being examined for their ability to catalyze the methylation of amino groups and the sulfation of amino groups. The former are N-methyltransferases that utilize S-adenosyl methionine as the methyl donor and the latter are N-sulfotransferases that use phosphoadenosyl phosphosulfate as sulfate donor.

Two N-methyltransferases have been purified to homogeneity from rabbit liver and differ in their specificity for aromatic amines. These enzymes are characterized by their broad substrate specificities which have overlapping elements. Present work is intended to answer questions bearing on the chemical mechanism of methyl group transfer to amines.

Sulfation of amines had been reported two decades ago but had never been confirmed despite many attempts to do so. The present studies with liver from guinea pigs reveals an enzyme capable of sulfating aryl amines (naphthylamine), alkylamines, and a secondary amine (desmethylimipramine). This work is in its early stages in which the enzymes involved are being purified.

K. Biochemical Lesions in Genetic mucopolysaccharidoses

Studies of a novel 3-O-sulfatase specific for a unique glucosamine residue found within a short antithrombin binding sequence of heparin have been extended. The enzyme has been demonstrated in plasma and in human leukocytes and has been partially purified from human urine. The enzyme has a molecular weight of approximately 65,000 and manifests three isoelectric points extending from pH 5.5 and 4.5. A heat-stable, macromolecular inhibitor is present in urine and coprecipitates with the enzyme in the presence of ammonium sulfate. The unique sulfated glucosamine residue is located near the non-reducing terminus of the heparin chain and this novel sulfatase may be essential for the metabolic degradation of heparin. The absence of this enzyme would lead to a variant of the Sanfilippo syndrome. To establish the specificity of the enzyme and to help identify the glucosamine sulfate residues found in heparin, a number of isomeric O-sulfated glucosamine derivatives have been prepared. These may serve as standards for establishing the structure of sulfated hexosamine residues found in oligosaccharides linked to anterior pituitary hormones and other glycoproteins.

L. Enzymes of Carbohydrate Metabolism in Invertebrates and Vertebrates

Limulus polyphemus hepatopancreas and muscle were examined for enzymes of hexose phosphorylation in order to demonstrate and characterize a fructomannokinase activity similar to that seen in the arthropod *Homarus americanus*. Unlike lobster, the predominant hexokinase of *Limulus* hepatopancreas is the fructomannokinase, which readily phosphorylates fructose and mannose but has little ability to phosphorylate glucose. The quantitatively large catalytic capacity for phosphorylation of fructose and mannose prompted an investigation of possible direct utilization of the reaction products by enzymes other than hexose-phosphate isomerases and glucose-6-phosphate dehydrogenase.

Preliminary surveys of homogenates of Limulus hepatopancreas have revealed the existence of an NAD+-specific hexose-phosphate dehydrogenase which does not react with glucose-6-phosphate but does react with mannose-6-phosphate and fructose-6-phosphate. Partial purification of this enzyme has been achieved by ion exchange and gel filtration HPLC chromatography. A possible alternate pathway for hexose phosphate metabolism may be operating in this multicular organism.

M. The Role of Free Inositol in Brain, Liver and Reproductive Organs

Myoinositol l-phosphatase is a pivotal enzyme in receptor-mediated calcium mobilization. First described in this laboratory, the immediate goal is its purification to homogeneity in order to explain three unusual features of the enzyme: its specificity for myoinositol phosphates, its optical non-specificity, and its specific inhibition by Li^+. The enzyme isolated from rat testis cytosol has been purified about 300-fold by a series of chromatographic steps including ion exchange and gel filtration HPLC.

Accumulating evidence linking lithium inhibition of this enzyme to the pharmacological role of lithium in brain disorders has led to the suggestion that toxic side effects of the drug are due to ^6Li and that pure ^7Li be used therapeutically. Determination in this laboratory of lithium toxicity in mice failed to show any difference either in the lethality of the two isotopes or in the inhibition of myoinositol l-phosphatase. The recommendation for this costly change in with lithium therapy is unjustified.

N. Thermodynamic and Kinetic Studies of Protein Structure and Enzymic Mechanisms

On exposure to acid pH, swine pepsinogen undergoes self cleavage to produce psuedo pepsin, by the loss of the first 16 residues in its sequence. Returning to neutrality, produces an irreversible conformational change in psuedo pepsin to a new globular form, which seems to be identical to the rapidly formed transient intermediate previously detected during the folding of intact pepsinogen. Like pepsinogen, but unlike nature psuedo pepsin, this neutral pH form of the protein can be reversibly unfolded by urea or high pH. The mechanism of the folding reaction, and the structure and stability of this neutral pH form of psuedo pepsin and the role of the first 16 residues in the mechanism of folding of pepsinogen are all under investigation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 17001-18 LBM

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanism of Receptor-Mediated Endocytosis in Hepatocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Gilbert Ashwell,	Medical Officer, LBM, NIADDK
	Joe Harford	Sr. Staff Fellow, LBM, NIADDK
	Djuro Josic	Guest Researcher, LBM, NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Biochemistry and Metabolism

SECTION
Section on Enzymes and Cellular Biochemistry

INSTITUTE AND LOCATION
NIADDK, LBM, Bethesda, MD. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The pathway and endocytosis of desialylated serum glycoproteins has been analyzed in terms of a series of sequential, compartmentalized stages. Thus, procedures are now available to facilitate the accumulation and/or recovery of vesicles at specific loci, in an attempt to understand the molecular details underlying the various segregation steps.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 17002-14 LBM

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Enzymatic Basis of Detoxication

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI: William B. Jakoby, Acting Chief, LBM, NIADDK
Sherry Ansher, Staff Fellow, LBM, NIADDK
Sengoda Ramaswamy, Visiting Associate, LBM, NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Biochemistry and Metabolism

SECTION
Section on Enzymes and Cellular Biochemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, MD. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	3	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Conjugation reactions involving the amine functional group is under investigation. The respective enzymes being purified and characterized are concerned with methylation and with sulfation.

Methylation involves S-adenosylmethionine as methyl donor with three enzymes from rabbit liver that can serve as N-methyltransferases. Substrates include aryl as well as alkylamines.

Sulfation of amino groups, catalyzed by enzymes from guinea pig liver, is being investigated with phosphoadenosylphosphosulfate or donor and a variety of alkyl and aryl amines, including a secondary amine, as acceptor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 17003-17 LBM

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Polysaccharides in Morphogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Enrico Cabib, Research Chemist, LBM, NIADDK

Mohinder S. Kang,	Staff Fellow, LBM, NIADDK
Martin L. Slater,	Executive Secretary, ERP/NIADDK
Paul Szaniszlo,	Guest Researcher, LBM, NIADDK
Hinda Zlotnik,	Visiting Fellow, LBM, NIADDK

COOPERATING UNITS *(if any)*

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Laboratory of Biochemistry and Metabolism

SECTION

Section on Enzymes and Cellular Biochemistry

INSTITUTE AND LOCATION

NIADDK, LBM, Bethesda, MD. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A study on the relative distribution of mannoprotein and glucan in the cell wall of Saccharomyces cerevisiae has shown that the mannoprotein forms an external layer that determines cell wall porosity. The linkage between the different components of the cell wall will be the subject of future studies.

The spatial controls that determine localization of the primary septum in yeast will be investigated with the help of a mutant that lays down septa at abnormal locations.

Chitin synthetase from S. cerevisiae has been highly purified by a novel procedure. Efforts are made to obtain antibodies against the enzyme to study its cellular localization and the relationship between chitin synthetase zymogen and active form. Experiments are also under way to clone the synthetase gene.

$\beta(1\rightarrow3)$Glucan synthetase, the enzyme that catalyzes the synthesis of the major structural polysaccharide in the Saccharomyces cell wall, has been studied in several fungi. The activation of the enzyme by nucleoside triphosphates was found to be conserved in species that are quite unrelated taxonomically. The kinetics of inhibition of the synthetase by the antibiotic, papulacandin, suggest that the enzyme exists in at least two different forms.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 17004-16 LBM

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Thermodynamic and Kinetic Studies of Protein Structure and Enzymic Mechanisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Peter McPhie, Research Chemist, LBM, NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Biochemistry and Metabolism

SECTION

Section on Enzymes and Cellular Biochemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, MD. 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1	1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

On exposure to acid pH, swine pepsinogen undergoes self cleavage to produce psuedo pepsin, by the loss of the first 16 residues in its sequence. Returning to neutrality, produces an irreversible conformational change in psuedo pepsin to a new globular form, which seems to be identical to the rapidly formed transient intermediate previously detected during the folding of intact pepsinogen. Like pepsinogen, but unlike native psuedo pepsin, this neutral pH form of the protein can be reversibly unfolded by urea or high pH. The mechanism of the folding reaction, the structure and stability of this neutral pH form of psuedo pepsin and the role of the first 16 residues in the mechanism of folding of pepsinogen are all under investigation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 17006-10 LBM

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Function of Bacterial Outer Membrane Components

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: John D. Foulds, Research Chemist, LBM, NIADDK

John Newland, Staff Fellow, LBM, NIADDK
Max Oeschger, Expert, LBM, NIADDK

COOPERATING UNITS *(if any)*

Loretta Leive, Research Chemist, LBM, NIADDK
Richard Silver, Research Chemist, BB/DBP

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Laboratory of Biochemistry and Metabolism

SECTION

Section on Enzymes and Cellular Biochemistry

INSTITUTE AND LOCATION

NIADDK, LBM, Bethesda, MD. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A class of E. coli outer membrane proteins called porins have been shown to have a role in the expression of K1 capsule biosynthesis. The rate capsule expression is markedly reduced in porin-negative strains. Newly synthesized K1 capsule was found only at the ends of an E. coli cell. This line lacks the capsule-associate porin protein K1.

The acrA mutation in E. coli results in hypersensitivity to a variety of antibiotics. The acrA gene has been cloned and the gene product identified as a component of the cytoplasmic membrane with a molecular weight of about 30,000.

The "extracellular" DNAse gene of V. cholerae has been cloned and the gene product identified, although the DNA'se is supposed to be an extracellular enzyme, we find more than 90% of the activity is associated with the cell.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 17007-03 LBM

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Receptor Regulation and Iron Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Richard Klausner, Medical Officer, LBM, NIADDK

Joe Harford, Senior Staff Fellow, LBM, NIADDK
Elena Mattia, Visiting Associate, LBM, NIADDK
Krishnamurthy Rao, Visiting Fellow, LBM, NIADDK
Jos van Renswoude, Vising Associate, LBM, NIADDK
Daniel Shapiro, Guest Worker, LBM, NIADDK

COOPERATING UNITS *(if any)*

Warner Green, M.D., Metabolism Branch, NCI

LAB/BRANCH

Laboratory of Biochemistry and Metabolism

SECTION

Section on Enzymes and Cellular Biochemistry

INSTITUTE AND LOCATION

NIADDK, LBM, Bethesda, MD. 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
7	7	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have demonstrated that the transferrin (Tf) receptor is a highly regulated molecule. It enters the cell through the classic pathway of endocytosis but only when activated by the appropriate signals. These can be given by either ligand or anti-receptor antibody or by activation or protein kinase C using phorbol esters. The latter result in a dramatic increase in receptor phosphorylation. Once inside the cell, ligand-driven receptors recycle to the plasma membrane. In contrast, antibody-driven receptor moves to the lysosomes wehre it is degraded.

Using normal peripheral blood human T cells, we have demonstrated that only an agonistic ligand such as IL-2 will stimulate the internalization of the T cell growth factor receptor. Anti-receptor antibodies with antigonistic activity are not internalized.

We have shown that the regulation of the human Tf receptor by iron occurs via alterations in the level of the mRNA for the receptor. The cells sense a very labile pool of iron and even the process of down regulating receptors results in a rapid enhancement of receptor biosynthesis. This may explain why all growing cells need to be continuously exposed to Tf. Consistent with the rapid sensing of iron by the genome, we find that a small percentage of iron released in the cell by Tf is rapidly delivered to the nucleus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 17008-01

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Organelle Biology - The structure, function and biogenesis of the Golgi

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Richard Klausner, Medical Officer, LBM, NIADDK
Ignacio Sandoval, Visiting Scientist, LBM, NIADDK
Juan Bonifacino, Visiting Fellow, LBM, NIADDK

COOPERATING UNITS *(if any)*

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Laboratory of Biochemistry and Metabolism

SECTION
Section on Enzymes and Cellular Biochemistry

INSTITUTE AND LOCATION
NIADDK, LBM

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Monoclonal antibodies have been raised against proteins of the Golgi apparatus. These are directed towards determinants present on integral membrane protein or peripheral and context proteins. Immunofluorescence microscopy has been used to characterize the morphologic distribution of the antigens recognized by each clone. Several distinct patterns of distribution have been seen. The specific antigen recognized of each monoclonal antibody is being determined by either immunoprecipitation of labelled proteins or immunoblotting. Several clones produce antibodies that recognize the carbohydrate moiety of Golgi glycoproteins. The topological localization with respect to the membrane of the epitopes recognized by the antibody are being determined. This library of monoclonal anti-Golgi antibodies will allow us to study the biosynthesis, degradation, dynamics and function of this critical organelle.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 18000-19 LBM

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Hormone-Dependent Development of Mammary Gland

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)*

PI: Yale J. Topper, Chief, Section on Intermediary Metabolism, LBM, NIADDK

Others: Lakshmanan Sankaran, Senior Staff Fellow, LBM, NIADDK
Piotr Chomczynski, Visiting Scientist, LBM, NIADDK
Colin Prosser, Visiting Fellow, LBM, NIADDK
Anil Balapure, Visiting Fellow, LBM, NIADDK

COOPERATING UNITS *(if any)*

Project No. Z01 CB 08218-07
P.K. Qasba, Research Chemist, National Cancer Institute

LAB/BRANCH

Laboratory of Biochemistry and Metabolism

SECTION

Section on Intermediary Metabolism

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
5.5	5.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We reported previously that insulin is essential for the accumulation of casein mRNA in explants of murine mammary tissue. Since the rate of accumulation of mRNA is a function of both the rate of its transcription and of its half-life, it was of interest to determine which component is influenced by insulin. Others had shown that prolactin, another hormone essential for the accumulation of murine casein mRNA, markedly increases the half-life of the mRNA and increases its transcription rate in the presence of insulin. Our studies confirm the marked effect of prolactin on the half-life of the mRNA, and demonstrate that insulin is not required for this effect. However, we have also observed that prolactin does not stimulate the transcription of the 25K mRNA gene in the absence of insulin. Although insulin has been shown previously to exert a negative influence on the transcription of the phosphoenolpyruvate carboxykinase gene, in liver, the mammary system may be the first known instance in which insulin has been demonstrated to exert an essential, positive influence on transcription.

We previously reported that estrogen-depletion renders mouse mammary cells incapable of casein gene expression in the presence of insulin, glucocorticoid and prolactin. These cells retain responsiveness to insulin and glucocorticoid, but lack responsiveness to prolactin. The inability to express casein genes was ascribed to a post-binding prolactin-related lesion. However, our recent results on mammary cells from estrogen-depleted rats casts a different interpretation on the role of estrogen in mammary differentiation. The estrogen-depleted rat cells have lost about 90% of their capacity for casein gene expression. They, too, retain full general insulin - and glucocorticoid - responsiveness. However, unlike the corresponding mouse cells, the estrogen-depleted rat cells also retain general responsiveness to prolactin. In the absence of the female sex steroid, rat mammary cells appear to lose only their unique responsiveness to the lactogenic hormones.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 18001-10 LBM

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Role of Free Inositol in Brain, Liver and Reproductive Organs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Frank Eisenberg, Jr., Research Chemist, LBM, NIADDK

Others: R. Parthasarathy, Visiting Fellow, LBM, NIADDK

COOPERATING UNITS *(if any)*

None

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NIADDK, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.5	2.0	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Myoinositol 1-phosphatase, a pivotal enzyme in receptor-mediated Ca^{2+} mobilization, was first described in this laboratory. During the course of purification of the enzyme we have shown that it is distinct from non-specific alkaline phosphatase by its narrow pH optimum (7-8), inhibition by Li^{+}, inertness toward levamisole, heat stability, and greater mobility on polyacrylamide gel electrophoresis. The function of the enzyme is the release of free myoinositol from enantiomeric myoinositol 1-phosphates from two sources: (1) isomerization of glucose 6-phosphate produces the L isomer in a de novo pathway that maintains in the organism the supply of free myoinositol for its primary role, the formation of phosphatidylinositol and its phosphates, and (2) receptor-mediated decomposition of phosphatidylinositol 4,5-bisphosphate releases D-myoinositol 1,4,5-trisphosphate and diacylglycerol, both recognized as second messengers in Ca^{2+} mobilization; the trisphosphate is then sequentially dephosphorylated to free myoinositol via D-myoinositol 1,4-bisphosphate and D-myoinositol 1-phosphate, a substrate for the enzyme under investigation. Free myoinositol from both sources is then recycled for renewed receptor activation. The purification of myoinositol 1-phosphatase to homogeneity is the immediate goal of this research necessary to explain three unusual features of the enzyme: (1) its specificity for myoinositol phosphates, its optical non-specificity, and its specific inhibition by Li^{+}. The enzyme isolated from rat testis cytosol has been purified about 300-fold by a series of chromatographic steps including ion exchange and gel filtration HPLC and is nearly homogeneous. Accumulating evidence linking Li^{+} inhibition of this enzyme to the pharmacological role of Li^{+} in brain disorders has led to the suggestion that toxic side effects of the drug are due to ^{6}Li and that pure ^{7}Li be used therapeutically. Determination in this laboratory of Li^{+} toxicity in mice failed to show any difference either in the lethality of the two isotopes or in the inhibition of myoinositol 1-phosphatase. The recommendation for this costly change in Li^{+} therapy is unjustified.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 18002-11 LBM

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on Lysosomal Cystine Transport

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Frank Tietze, Research Chemist, LBM, NIADDK

COOPERATING UNITS *(if any)*

William A. Gahl, Section on Human Biochemical and Developmental Genetics,
National Institute of Child Health and Human Development

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TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The enzymic hydrolysis of proteins within lysosomes of animal cells produces free amino acids which normally transit the lysosomal membrane to the exterior milieu at a rapid rate. In studies conducted over the past several years we have obtained evidence that transport of the amino acid cystine from lysosomes of normal human polymorphonuclear leucocytes occurs by way of a stereospecific, carrier-mediated process exhibiting saturation kinetics. No exodus of cystine can be detected from corresponding lysosomes isolated from patients afflicted with the inherited childhood disorder cystinosis, a potentially fatal disease characterized by excessive storage of cystine, but of no other amino acid, within the lysosomes of many tissues. The maximum velocity of cystine egress from lysosomes of obligate heterozygotes for cystinosis was found to be one-half that of normal, indicating that clinically unaffected carriers possess half the normal complement of the putative cystine carrier. These observations constitute presumptive evidence for a defective lysosomal cystine carrier as the underlying cause of human cystinosis.

More recent studies have been directed to the further characterization of the lysosomal cystine carrier system. It has been found that cystine egress from normal polymorphonuclear leucocytes is stimulated by ATP in the presence of potassium ions. This stimulation is not affected by agents known to block the ATP-dependent lysosomal proton pump (e.g., N-ethylmaleimide, ammonium ions or the protonophore CCCP) and thus appears to be due to a mechanism other than acidification per se, possibly to phosphorylation of the cystine carrier.

PHS 6040 (Rev 1/84) GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 18003-11 LBM

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of the Hormone-dependent Growth and Differentiation of the Mammary Gland

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory and institute affiliation)*

PI:	T. Oka	Senior Investigator	LBM, NIADDK
Others:	S. Okamoto	Visiting Fellow	LBM, NIADDK
	M. Taga	Visiting Fellow	LBM, NIADDK
	F. Cossu	Visiting Fellow	LBM, NIADDK
	M. Arakawa	Guest Worker	LBM, NIADDK
	J. Perry	Research Biologist	LBM, NIADDK

COOPERATING UNITS *(if any)*

None

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INSTITUTE AND LOCATION

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TOTAL MAN-YEARS	PROFESSIONAL	OTHER
6.5	5.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Using a primary mammary epithelial cell culture system, we found that epidermal growth factor (EGF), a peptide hormone synthesized and secreted by the salivary gland, stimulated mammary cell proliferation and inhibited mammary cell differentiation. These effects of EGF were apparent at the physiological concentrations.

The mRNA sequence of whey acidic protein, a major mouse milk protein, was cloned in full length. The sequence analysis of the cDNA clones revealed that the mRNA contains a 28 nucleotide-long 5'-noncoding region. Three different portions of this region were identified as possible sites of interaction with the 3'-end of 18S rRNA that may facilitate efficient rate of translation of the mRNA.

Cortisol 21-mesylate was previously shown to exert an anti-glucocorticoid action in rat hepatoma cell culture. When this agent was examined in organ culture of mouse mammary gland, it produced a glucocorticoid-like effect in stimulating milk protein synthesis. The potency of cortisol mesylate was about 1/10 to 1/30th of that of cortisol. These results indicate that the effect of cortisol mesylate can be agonist-like or antagonist-like, depending on its target tissues.

The differentiative potential of the lactating mouse mammary epithelial cells were examined in the organ culture system. The data indicate that in the presence of insulin, cortisol and prolactin mammary epithelial cells in culture first lose and then regain the ability of synthesizing milk protein without requiring the formation of new daughter cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 18004-11 LBM

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical Lesions in Genetic Mucopolysaccharidoses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)*

PI: Irwin G. Leder, Research Chemist, LBM, NIADDK

COOPERATING UNITS *(if any)*

None

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies of a novel 3-O sulfatase specific for a unique glucosamine residue found within a short antithrombin binding sequence of heparin have been extended. The enzyme has been demonstrated in plasma and in human leukocytes and has been partially purified from human urine. The enzyme has a molecular weight of approximately 65,000 and manifests three isoelectric points extending from pH 5.5 and 4.5. A heat-stable, macromolecular inhibitor is present in urine and coprecipitates with the enzyme in the presence of ammonium sulfate. The unique sulfated glucosamine residue is located near the non-reducing terminus of the heparin chain and this novel sulfatase may be essential for the metabolic degradation of heparin. The absence of this enzyme would lead to a variant of the Sanfilippo syndrome. To establish the specificity of the enzyme and to help identify the glucosamine sulfate residues found in heparin, a number of isomeric O-sulfated glucosamine derivatives have been prepared. These may serve as standards for establishing the structure of sulfated hexosamine residues found in oligosaccharides linked to anterior pituitary hormones and other glycoproteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 18005-11 LBM

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
B Cell Proliferation: Mechanism of Triggering Activation and Fate of Receptors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Milton Kern, Research Chemist, LBM, NIADDK

Others: Madduri Ramanadham, Visiting Associate, LBM, NIADDK
Blossom Johnson, Laboratory Assistant, LBM, NIADDK

COOPERATING UNITS (if any)

None

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TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.5	2	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Splenic B cells are induced to proliferate upon culture with antibody having specificity for surface membrane immunoglobulins. Cells treated with pronase, washed and then cultured with antibody, exhibited a greater than 5-fold enhancement of DNA synthesis whereas pronase treatment, per se, was not mitogenic. Cells from immature mice (2 weeks of age) which essentially do not show a proliferative response to antibody become responsive subsequent to pronase treatment. In addition, splenocytes derived from strain CBA/N (x-chromosome linked immunodeficient mice) do not synthesize DNA in response to anti-mouse IgM although such cells respond readily to anti-IgM covalently linked to Sepharose. However, after pronase treatment, CBA/N splenocytes responded to free anti-IgM at 40 to 100 percent of the DNA synthetic capacity detected with Sepharose linked anti-IgM. Murine B lymphocytes in the presence of anti-IgM begin to synthesize DNA at about the 36th hour of culture although the onset of synthesis in response to other B cell reactive mitogens occurs at approximately 18 hours. By contrast, the onset of DNA synthesis by pronase treated cells in response to anti-IgM required only 18 hours. As judged from a variety of experiments pronase functions by attacking surface sites on B cells as a consequence of its proteolytic properties. Taken together the findings suggest the interpretation that a pronase sensitive site on the surface of B cells functions to regulate the proliferative response resulting from perturbation of the surface immunoglobulin receptors.

Enhanced phosphorylation of protein in membranes of cells cultured with anti-IgM linked to Sepharose was observed initially at about 16 hours of cell culture and continued thru the 48th hour yielding the same time course as for 3H-thymidine incorporation. However, the bulk of the enlargement of cells (blast formation) occurred prior to 16 hours of culture i.e., before detection of enhanced phosphorylation or DNA synthesis. These findings suggest the possibility that membrane protein phosphorylation may play a crucial role during the committment for entry of cells into S phase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 18006-13 LBM

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Enzymes of Carbohydrate Metabolism in Invertebrates and Vertebrates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Paul K. Goldsmith, Research Biologist, LBM, NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Biochemistry and Metabolism

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Limulus polyphemus hepatopancreas and muscle were examined for enzymes of hexose phosphorylation in order to demonstrate and characterize a fructomannokinase activity similar to that seen in the arthropod Homarus americanus. Unlike lobster, the predominant hexokinase of Limulus hepatopancreas is the fructomannokinase, which readily phosphorylates fructose and mannose but has little ability to phosphorylate glucose. The quantitatively large catalytic capacity for phosphorylation of fructose and mannose prompted an investigation of possible direct utilization of the reaction products by enzymes other than hexose-phosphate isomerases and glucose-6-phosphate dehydrogenase.

Preliminary surveys of homagenates of Limulus hepatopancreas have revealed the existance of an NAD^+-specific hexose-phosphate dehydrogenase which does not react with glucose-6-phosphate but does react with mannose-6-phosphate and fructose-6-phosphate. Partial purification of this enzyme has been achieved by ion exchange and gel filtration HPLC chromatography.

The significance of this project lies in the identification in a multicellular organism of a possible alternate pathway for hexose phosphate metabolism.

ANNUAL REPORT OF THE LABORATORY OF CHEMISTRY
National Institute of Arthritis, Diabetes, & Digestive & Kidney Diseases

SECTION ON BIOCHEMICAL MECHANISMS

FLUORO ANALOGS OF ENZYME SUBSTRATES:

As a result of our development of a photochemical route to ring-fluorinated imidazoles, a wide variety of fluoro analogs of biologically significant imidazoles have become available for biological investigation. For example, 2-fluoro-L-histidine not only is incorporated in microbial and animal protein in place of histidine, but also serves as an antibacterial, antiviral and antileukemic agent. Recent studies with labelled amino acids reveal that this analog also limits the uptake of several other amino acids into the cell. These, and other, biological properties appear to be very specific for the 2-fluoro isomer. It is noteworthy that, although the 4-fluoro isomer of the amino acid shows no significant biological activity, the same analog is quite useful in peptide linkage.

4-Fluoro-Im-TRH fails to bind to rat pituitary cells in vitro and fails to stimulate prolactin release in that system. On the other hand, microinjection into the rat hypothalamus results in strong elevations in heart rate and blood pressure, comparable to those produced by TRH itself; furthermore, blood levels of prolactin are similar to those found with TRH injection. Thus, the fluoro analog has finally demonstrated that the pituitary and CNS activities of TRH involve different receptors and that prolactin release can be effected outside of the pituitary. There are preliminary indications that the fluoro analog is also more resistant to enzymatic breakdown than is TRH. Efforts to synthesize 2-fluoro-Im-TRH have, thus far, been thwarted by the instability of synthetic intermediates. [Labroo, Fuerstein, Cohen]

Our earlier studies had shown 4-fluoroimidazole-5-carboxamide riboside to be a potent antiviral agent in cell culture systems. Furthermore, it had been demonstrated that, in human blood, the aglycone undergoes ribosylation and the riboside is phosphorylated by ATP to a triphosphate. Since the final product could not continue along the pathway to form an adenine ring, the stage of the metabolic block seemed to be well specified. We wished, therefore, to extend our studies to include the ribosides of 2,4-difluoroimidazole-5-carboxamide and of isomeric amino fluoroimidazoles. Fluoroimidazoles are obtained by photochemical replacement of amino groups, and aminoimidazoles are generally prepared by hydrogenolysis of arylazoimidazoles. Although the procedures work smoothly for a variety of imidazoles, the hydrogenolysis of an arylazoimidazole containing an additional fluoro or amino substituent results in the removal of the latter group. This obstacle to synthesis has been overcome by the application of a unique reducing agent, formamidine-2-sulfinic acid. Thus, 4-fluoro-2-amino-imidazole-5-carboxamide was prepared and was converted into the 2,4-difluoroderivative. Surprisingly, the isomeric 2-fluoro-4-aminoimidazoles are far less stable and must be stored at very low temperatures. The N-trifluoroacetyl derivatives, however, are quite stable in the solid state at 25°C. When dissolved at pH 7.4 and 37°C, these compounds lose the trifluoroacetyl group spontaneously ($t_{1/2} = 3$ hr) and the potential antiviral agents are released in situ. In parallel with the broad-spectrum antiviral activities already shown by 4-fluoroimidazole-5-carboxamide ribotide, the new compounds will also be investigated for such activities. Since these compounds may not survive further chemical manipulations, the ribosylation of the imidazoles by enzymatic means is being explored. 2,4-Difluoroimidazole-5-carboxylic ester was planned as a precursor to 2,4-difluorohistidine. To date, every attempt to reduce the ester function to an alcohol has resulted in loss of fluorine at C-2. Current approaches involve reduction of the ester prior to introduction of fluorine at C-2.

Biological studies with 2-fluoro-L-histidine have required the availability of the radiolabelled material. Although 4-tritio-2-fluoro-L-histidine is easily prepared by exchange with tritiated water, the label is not completely stable and the level of radioactivity is inadequate for some studies. A new approach has been achieved in which the dehydroamino acid is synthesized and is then hydrogenated to the DL-amino acid with tritium gas.

CHEMISTRY OF SUBSTITUTED IMIDAZOLES:

The remarkable antimetabolic activities of 2-fluorohistidine, as well as the inactivities of the 4-isomer, provided an impetus to develop synthetic methods for other new analogs of histamine and histidine with substituents at C-2, C-4 or both. We now have in hand analogs containing halogen, amino, formyl, cyano, nitro, ester, azido and trifluoromethyl groups. The azido, analogs may be useful for photoaffinity labeling of receptors and enzymes. [Jerussi, Phillips, Cohen]

Synthesis of 2-halohistidines proved especially difficult; a general procedure has now been developed in which 2-diazohistidine is decomposed in the presence of halide ion and copper powder or cuprous salts. Quite surprisingly and in spite of steric considerations, 2-iodohistidine is active against organisms which are unaffected by 2-fluorohistidine. [Labroo, Kirk, Cohen]

Ring-trifluoromethylated imidazoles show the unique property of losing hydrogen fluoride above pH 8 to form metastable difluorodiazafulvenes, which then react with any available nucleophile to form new covalent bonds. Such intermediates, derived from trifluoromethylhistamine or histidine, may be able to serve as covalent affinity labels for specific binding sites, both *in vitro* and *in vivo*. It would be desirable, therefore, to have available a series of trifluoromethyl analogues with a range of reactivities, and to be able to correlate reactivity with some substituent parameter. Our discovery of a simple photochemical method for the trifluoromethylation of imidazoles has made available a large series of analogues for study. We have now found that the reactivities of some members of the group can be correlated with the special electronic effect of certain substituents (capable of hyperconjugation or back-bonding). Computer analysis of reactivity data for a series of trifluoromethylimidazoles has provided a linear free energy relationship in which log k_r correlates with both inductive and resonance components of the respective substituents. According to computer- based predictions, the fluoro group would provide the ideal combination of acidity and reactivity under physiological conditions. We have, therefore, developed procedures for sequential photochemical introduction of fluorine and trifluoromethyl into imidazoles and have verified the predicted reactivities. We are now involved in the preparation of peptide hormones containing these substituents. Photochemical introduction of the trifluoromethyl group has been found practical for more complex imidazoles and studies are under way for the synthesis of the trifluoromethyl analog of the anti-ulcer drug, cimetidine. [S. Cohen, L. Cohen]

The trifluoromethyl analogs are labile in mildly alkaline media, making them potentially useful as receptor affinity labels for use in vivo. Toward this end, the isomeric trifluoromethylhistidine analogs of TRH have been synthesized and are undergoing biological evaluations. Although 2-CF_3-His-TRH fails to bind to pituitary cells in vitro and fails to release prolactin in this system, the compound does release prolactin in vivo; this result suggests that the brain contains nonpituitary receptors for TRH which also stimulate prolactin release. The use of TRH for diagnosis of hypothalamic dysfunction is limited by its powerful cardiovascular effects--large increases in heart rate and blood pressure. The 2-CF_3 analog is 5-6 times as effective as TRH in promoting prolactin release but has no significant cardiovascular effects--thus making it a potentially valuable diagnostic reagent. [Labroo, Feuerstein, Cohen]

The valuable properties of nitroimidazoles as radiation sensitizers and as selective cytotoxic agents for cancer treatment have stimulated considerable research into mechanisms of action and metabolic fate of the drugs. One theory holds that the nitroimidazole is reduced in vivo to the hydroxylaminoimidazole, which functions as a supernucleophile in cleaving phosphate ester bonds and in degrading polynucleotides. Efforts in many laboratories to synthesize hydroxyaminoimidazole have failed, since reduction of the nitro compounds leads directly to the amino analogs. Our discovery of a unique photochemical route to 2-fluoroimidazoles makes available an alternative approach to the 2-hydroxylaminoimidazoles, since fluorine is the only C-2 substituent known to be capable of nucleophilic displacement. Thus the reaction of 1-methyl-2-fluoro- imidazole with hydroxylamine does lead to the hydroxylamine derivative; unfortunately, the reaction continues with addition of a second molecule of hydroxylamine to the 4,5-double bond. This phenomenon is known to be a general property of imidazoles which contain a tautomerizable group in the 2-position-nucleophilic addition to the 4,5-double bond now becomes possible. An alternative approach has been developed: reduction of the nitroimidazole with zinc and ammonium chloride. 1-Benzyl-4-hydroxylaminoimidazole has recently been obtained in pure solid form; in contrast to claims of previous investigators, the compound is not unstable and exists as the nonaromatic oxime tautomer. Studies are in progress on its behavior with polynucleotides. [Takahashi, Cohen]

Exposure of 4-trifluoromethylhistamine or histidine to mild base or ammonia results in facile cyclization to deaza analogs of purines. Several compounds in this series have recently been found to have potent antiviral properties and our analogs are now being evaluated. Deoxy analogs of these compounds are also being incorporated into peptide hormones as rigid, cyclic analogs of histidine. [Labroo, Cohen]

CHEMISTRY, BIOCHEMISTRY AND PHARMACOLOGY OF BIOINDOLE ANALOGS:

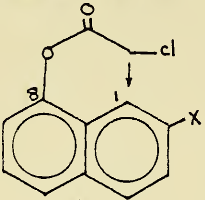
Tryptophan is an essential amino acid, serving as the precursor of the neurotransmitter, serotonin, and of the hormone, melatonin, in addition to its roles in enzymes and in receptor proteins. Tryptophan is metabolized in mammals by a pyrroloxygenase in the liver, where it can serve as a precursor of nicotinamide (Vitamin B_6) in some animals. In other tissues, tryptophan and related indoles are metabolized by a distinct oxygenase, the activity of which is dramatically increased (up to 100-fold) upon administration of bacterial lipopolysaccharides or interferon. The role of this oxygenase in the response of the organism to infection is unknown, however. We anticipated that certain 2-substituted tryptophans might serve as selective "suicide substrates" for these oxygenases. Analogs of tyrptophan with electronegative substituents at C-2 had not been previously prepared. We have obtained 2-chloro- and 2-bromo-L-tryptophan by radical halogenation, and 2-nitro-L-tryptophan as a minor product of direct nitration. The mechanisms of hydrolysis of the 2-halotryptophans at low pH have now been fully elucidated and reveal the involvement of intramolecular proton transfer in the conversion of the stable indole to the labile indolenine tautomer. An enzyme carboxyl group should also promote indolenine formation, suggesting the indolenine to be the true substrate for tryptophan enzymes. Indeed 2-oxindolyl-L-alanine and 2,3-dihydrotryptophan are excellent competitive inhibitors of these enzymes. This unique catalytic power of the carboxyl is now being utilized to catalyze the replacement of the 2-halo group by other nucleophiles in aprotic solvents. The availability of the novel 2-nitrotryptophan now provides an alternative route to other 2-substituted tryptophans--of potential value as affinity and photoaffinity labels, and as antibacterial agents. 5-Azido-L-tryptophan has already been found effective as a photoaffinity label for tryptophan synthase. The properties and hydrolysis mechanisms of 2-haloindole-3-acetic acids contrast markedly with those of the analogous propionic acids. [Phillips, Cohen]

GENERAL PRINCIPLES OF ENZYME CATALYSIS AND SIMULATION:

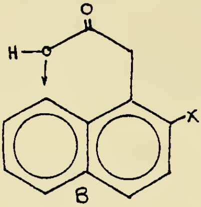
In order to account for the remarkable catalytic power of enzymes, it is generally considered that the activation free energy (the energy hill which must be surmounted to get from starting material to product) is contributed both by binding of the substrate to the enzyme (step 1) and by chemical manipulation of the bound substrate (bond-making and breaking, step 2). Popular opinion holds that most of the activation energy is supplied in step 2: We have proposed, however, that the overall catalytic process can be explained more reasonably if it is assumed that the first step (binding) contributes a more significant, and sometimes major, share of the activation energy. To support this theory, we have synthesized a large variety of test-tube models which simulate the bound substrate by being frozen into a single, favorable conformation and by having the interacting groups brought into the closest possible juxtaposition (stereopopulation control). The compounds undergo intramolecular reactions at rates comparable to those catalyzed by enzymes, sometimes even too fast to measure. Enzymes catalyze many reactions which cannot be observed under mild laboratory conditions. We have shown that our "locked" test-tube analogues can undergo a number of these reactions under physiological conditions of temperature and pH. Thus, one can demonstrate such difficult processes as hydride transfer and displacement of aromatic halogens.

The protein raises both the entropic and enthalpic components of the free energy content of the substrate by binding it in a single, rigid conformation. To prove that our model compounds are truly frozen in unique conformations in solution, we have used temperature-variable NMR studies, x-ray crystallography, computer calculations of free energy content and kinetic analysis. Thus, we have found that log k for enhanced cyclization is a function of the Van der Waals size of restricting groups and is independent of electronic effects. More recently, we have initiated efforts to demonstrate that these molecules possess molecular chirality, and that the resolved enantiomers do not racemize at elevated temperatures. Indeed, some of our models show such slow rotation of single bonds (half-life = 20 minutes), that conformational change becomes the rate-limiting step in cyclization.

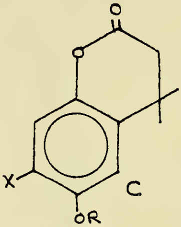
A number of our studies on rigid conformational systems have involved interactions between positions 1 and 8 of the naphthalene nucleus (the peri bridge). Synthesis of simple compounds in this series proved straightforward; more complex members have defied classical synthetic approaches. We are now involved in the development of new nonclassical approaches (photochemical, electrochemical, radical) to achieve the required ring closures (A and B).



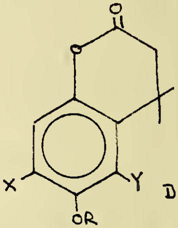
A



B



C



D

Seven examples of series C have been synthesized in order to evaluate the electronic effect of X on the ^{1}H and ^{13}C NMR signals of the various ring nuclei, as well as on the infrared frequency of the carbonyl group. The data have proved exceedingly complex to analyze and a logical electronic pattern has not yet been found. Furthermore, the pattern of infrared frequencies for the carbonyl group suggests that the electronic effect of X is transmitted neither through space nor through the shorter sigma-bond pathway (via the ring oxygen), but through the longer saturated carbon chain. Several other members of this series have defied synthesis, and efforts are continuing. A number of members of series D have also been synthesized in order to evaluate the steric effect of Y on the gem-dimethyl group of the saturated lactone ring. We have already shown that log k for lactone formation is a linear function of the van der Waals radius of Y and are attempting to extend this series for further confirmation. Synthesis in series D is rendered difficult in that the crowded environment of Y will not permit occurrence of any mechanism which involves a tetrahedral intermediate. Thus, a new tool has been discovered to prove the existence or nonexistence of tetrahedral intermediates in certain reaction mechanisms.

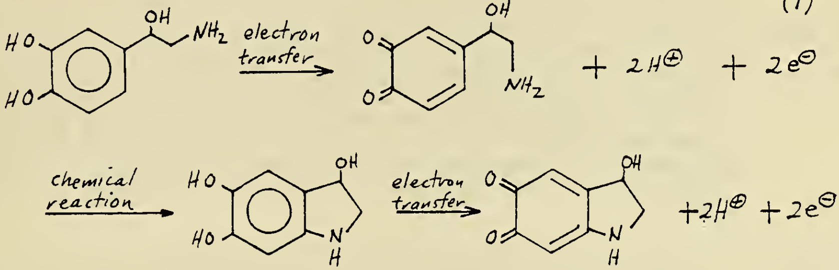
The lactone rings of series D are not flat and are, therefore, capable of molecular chirality. We believe that, if Y is sufficiently large, inversion of the lactone ring will be prevented and enantiomers should be isolable. To this end, we have prepared chromatographic columns coated with optically active charge transfer reagents and are attempting to resolve compounds of series B by charge transfer interaction. [King, Cohen]

HALOGENATED BIOGENIC AMINES IN BIOCHEMISTRY AND PHARMACOLOGY:

Work has continued on the chemistry and biology of ring-fluorinated analogs of biogenic amines. As reported previously, MNDO calculations show that fluorine substitution on the aromatic ring of norepinephrine (NE) reduces the residual electronic in charge on the carbon to which it is bound by about 0.25 eu. We have suggested that such a reduction in electron density may play a role in the fluorine-induced adrenergic agonist specificities of F-NE's. These electronic pertubations caused by fluorine are being explored further using chemically induced nuclear magnetic polarization (CIDNP), a technique which will produce an experimental measure of changes in electron densities of individual carbons of the aromatic ring. [K. Muszkat (Weizman Institute of Science), K. Jacobson, K. Kirk]

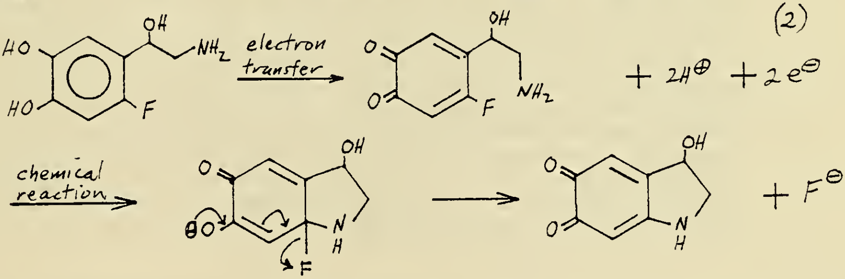
FNE's and fluorinated dopamines (FDA) show the expected tendency to undergo oxidation in solutions of pH values equal to or higher than the phenolic pKa. Qualitatively, the 6-fluoro catecholamines have appeared to be more prone to oxidation than the parent or the 2- or 5-fluoro analogs. Since the oxidation of catechlamines and catecholamino acids has biological importance with respect to adrenochrome and melania production, we have studied the effects of fluorine substitution on the oxidation of NE and DA using cyclic voltammetry. In the NE series, the ease of oxidation, as determined by half-wave potentials, was in the rank order 6FNE > 5FNE > 2FNE > NE, this result consistent with our qualitative observatins. A significant difference in the mechanism of oxidation was also observed. The oxidation pathway of catecholamines such as NE follows what is known as the ECE reaction path, i.e., an electron transfer (oxidation), followed by chemical reaction, followed by another electron transfer (Scheme 1).

(1)



Under appropriate reaction conditions (unprotonated amine, slow scan rate), the transfer of 4 electrons can be seen in the oxidation of NE. In the cyclic voltammogram of 6-FNE, a maximum of two electrons are transferred. Furthermore, during oxidation, fluoride ion is released (determined by an $F^{\ominus}$ specific ion electrode). We propose the mechanism shown in Scheme 2 to explain these results.

(2)



5-FNE appears to follow the ECE mechanism while 2-FNE apparently follows both pathways (this implies competing cyclization to the 2-position in the second step). The results of a similar study of DA and 2-, 5-, and 6-FDA were qualitatively similar. [M. Rice (Kansas); C. R. Creveling (LBC), K. Kirk]

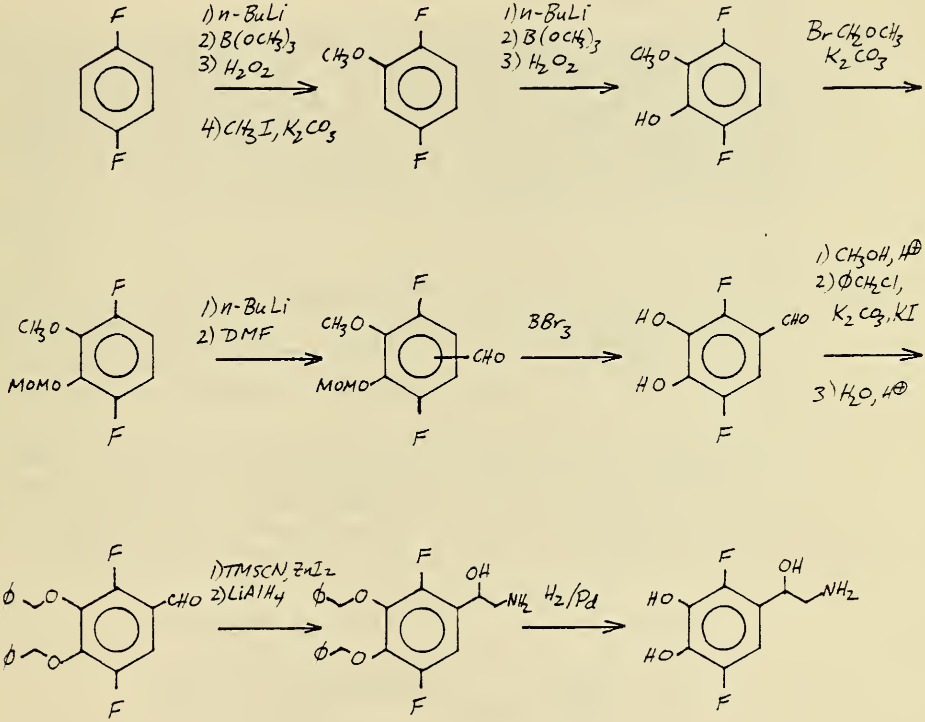
The biological implications of the oxidation initiated release of fluoride from 6-F-catecholamines (and presumably from the corresponding amino acids) is being explored. Of particular interest is the potential in vivo toxicity to cells undergoing rapid melanin synthesis.

Our previous work, has shown that 6-fluorocatecholamines behaved as false adrenergic neurotransmitters and thus should have potential as ^{18}F-PET scanning agents. Subsequently, Firnaw, et. al. (McMaster University) have shown accumulation of radioactivity, detected by PET scanning, in the caudate nucleus of human brain following administration of ^{18}F-6-FDOPA. Because of the concentration of dopaminergic neurons in this brain region, these workers assumed that the accumulation of radioactivity reflected accumulation of ^{18}F-6-F-DA. We have investigated the nature of the radioactive species concentrating in this brain region of rats using tritium labeled 6FDOPA and HPLC analyses of metabolite formation as a function of time. In the caudate nucleus, tritium counts were present associated with 6-FDOPA, 6-FDA, 6-F-dihydroxyphenylacetic acid (6-FDOPAC), 3-methoxy-6-FDOPA, and 6-F-homovanillic acid (6-FHVA) (authentic samples of all metabolites were synthesized unambiguously). The major peak accumulating was 6-FDA (about 60%) reaching a maximum level after 30 minutes. Since there is no significant accumulation of 3-methoxy-6-FDOPA, it is reasonable to postulate that the radioactivity detected in the human brain by ^{18}F-PET scans is, in fact, due to accumulations of ^{18}F-6-FDA. [C. Chieuh (NIM), K. Kirk]

An important feature of the use of ^{18}F-6-FDOPA, rather than ^{18}F-5FDOPA, fo PET scanning is that the 6-F catechol series appears to be metabolized more slowly by catechol O-methyl transferase (COMT). We are continuing our study of the mechanism of COMT catalyzed methylation of catechols using fluorine substitution as a mechanistic probe. This study involves qualitative HPLC analyses of reaction mixtures, with product identification based on comparison with authentic fluorinated normetanephrines and norparanephrines. These were synthesized from the corresponding vanillins and isovanillins by the usual TMSCN/ZnI$_2$-LAH side chain elaboration. We previously reported that the course of methylation of NE varies with pH and that the absolute amount of 4-O-methylation increases with pH with an apparent reaction pKa of ∿8.5. To determine if this reflects ionization of the substrate (phenolic pKa ∿8.5) or of a functional group on the enzyme, we have studied the pH effects on the methylation of 5-FNE (phenolic pKa = 7.9). As observed in earlier work, the relative amount of 4-O-methylation is significantly greater in the 5-F-series. The absolute amount of 4-O-methylation increases with pH to pH ∿7.9, then decreases. The absolute amount of 3-O-methylation decreases with pH, again with an apparent pKa of ∿7.9. We have yet to make a complete interpretation of these results, although they clearly indicate phenolic ionization alters the meta/para methylation ratio. We are extending this study to include 2F- and 6-FNE. [D. Thakker (NCDB), C. R. Creveling (LBC), K. Kirk]

The dramatic effects of fluorine on the adrenergic agonist specificities of NE have spurred our interest in preparing the more difficultly accessible difluoro-NE's, viz. 2,5-DiFNE, 2,6-DiFNe, and 5,6-DiFNE). The first of these, 2,5-DiFNE, has now been prepared by the route shown in scheme 3. Phenolic groups were introduced into p-difluorobenzene by literature procedures. Becaus polyfluorination renders electrophilic ether cleavage very difficult, the more labile methoxymethyl group was introduced to protect the second hydroxyl group during the third lithiation sequence. After formylation, BBr$_3$ deblocking and benzylation gave the key intermediate, 3,4-dibenzyloxy-2,5-difluorobenzaldehyde, a compound which will be used to prepare a series of 2,5-difluorocatecholamines and amino acids. 2,5-DiF-NE was prepared by the usual TMSCN mediated elaboration ofthe side chain and hydrogenolytic debenzylation. Biological studies with a number of adrenergic systems are in progress. [G. Chen, K. Kirk).

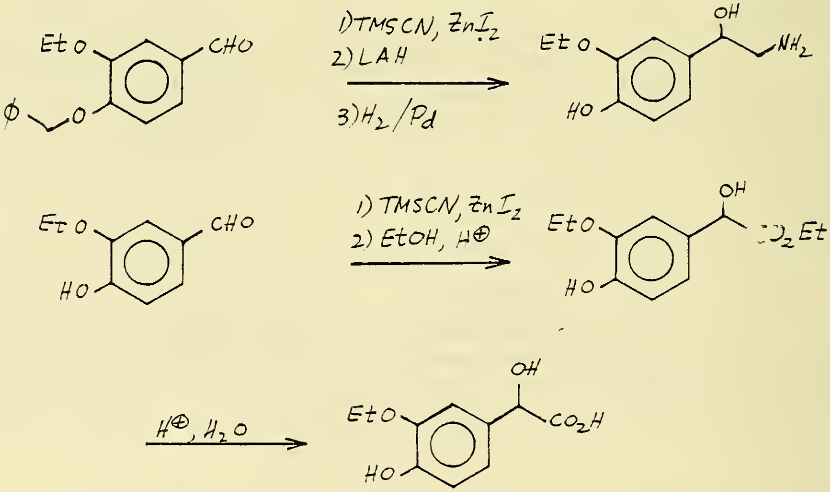
(3)



In our research on the biology of ring-fluorinated biogenic amines, we have frequently relied on HPLC analyses of biological samples or reaction products, using as HPLC standards compounds prepared by unambiguous synthetic routes. A related technique for HPLC analyses of endogenous metabolites, including material present in extremely low concentrations in clinical samples, has been developed using "fluologs" and homologs as standards. Compared to the endogenous compound, the standards should have a similar, but not identical, retention time, should have essentially identical behavior in chemical manipulations, and should have comparable sensitivity in the HPLC detection system. We previously reported success in the simultaneous determination of 3-methoxy-4-hydroxyphenylglycol (MHPG), 5-hydroxyindolacetic acid (5HIAA), and homovanillic acid (HVA) in cerebral spinal fluid (CSF) using 5-FHVA and 3-ethoxy-4-hydroxyphenylglycol (EHPG) as standards. A similar procedure has now been developed for the detection of urinary MHPG in man by HPLC and electrochemical detection using EHPG as a standard. [R. T. Brown, J. Oliver, I. Kopin (NIMH); K. Kirk]

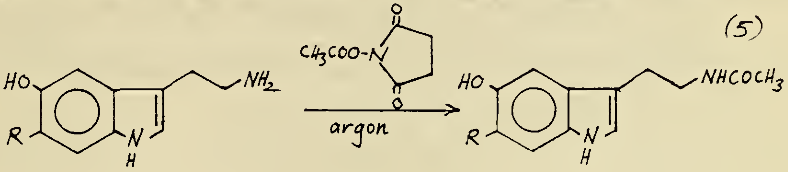
The success of these procedures (request for standards from clinical and research laboratories world-wide have been received) have prompted synthetic efforts to prepare suitable standards for other endogenous amines and metabolites, particularly those related to neurochemical processes and/or disease states. Thus, homologs of normetanephrine and of vanillylmandelic acid have been prepared (Scheme 4), and have proven to be extremely effective as standards for the quantitation of these metabolites.

(4)



A different strategy was developed for a highly sensitive method of detection for 5-hydroxytryptamine (5HT). While 6-F5HT has been an effective HPLC standard, difficulties from biological samples in extraction and instability of 5HT thwarted efficient reliable quantitation. A combination of acylation with N-succinimidyl acetate, inert gas purging, and the use of 6F5HT as standard produced the first convenient, reliable, and sensitive method for quantitation of 5HT in the CSF (down to < 10^{-12} mole) (Scheme 5).

(5)

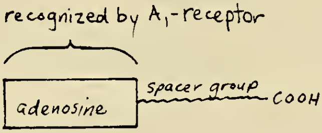


The detection of histamine by HPLC using electrochemical detection presents an additional problem in that the oxidation potential of this amine is too low to permit its detection at a reasonable detector voltage. We have overcome this problem, and at the same time increased extraction efficiency by acylating the primary amine with a reagent containing an easily oxidized phenolic moiety. In this case, 4-chlorohistamine is used as standard. This combined strategy of increasing solubility and sensitivity to detection by a simple acylation procedure clearly has broad potential for the detection of other amines, particularly where solubility and/or electrochemical detection presents difficulties.

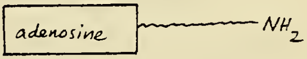
The development of these standards and procedures should widen the scope of HPLC coupled with electrochemical detection as an inexpensive, reliable, and convenient technique for quantitative analyses of biological extracts. [M. Linnoila, T.Brown (NIAAA); K. Jacobson, K. Kirk]

FUNCTIONALIZED CONGENERS OF BIOACTIVE COMPOUNDS:

Based on published structure activity relationships, we predicted that the 6-amino position of the adenine ring of adenosine could accomodate a covalently-attached functionalized chain without the loss of biological activity. A series of functionalized carboxylic (1) and amino (2) congeners based on N^6-phenyl and simple derivatives such as amides were found to be active in competitive binding assays on the A_1-receptor of the rat cerebral cortex.



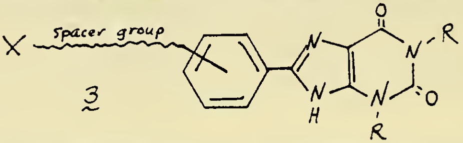
1



2

The affinity for the receptor was highly dependent on the structure of the spacer group and the molecule to which the functionalized adenosine was attached. The affinity constants in the competitive displacement of the radioactive ligand CHA (N^6-cyclohexyladenosine) ranged from 10^{-9} to greater than 10^{-7} molar. Size of the attached group was not found to be a limiting factor, thus we feel that these functionalized congeners of adenosine will have a variety of applications as molecular probes. [K. Jacobson, K. Kirk, W. Padgett (LBC), J. Daly (LBC)]

A series of functionalized antagonists to the same receptor, based on 8-phenyl theophylline was synthesized and found to be active in binding studies. The receptor affinity was particularly strong when the 1,3-dipropyl modification (previously reported by J. Daly and coworkers) was included.



3

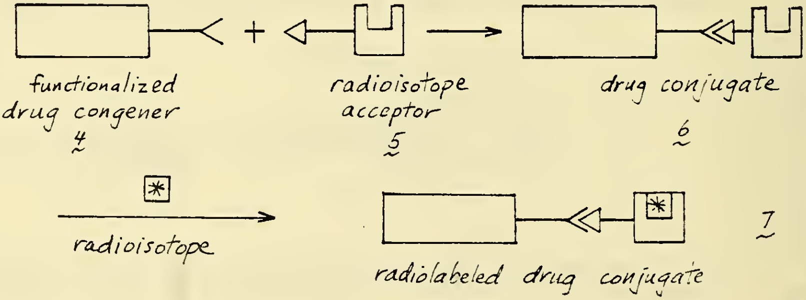
R = alkyl

X = NH_2 or COOH

Moreover, the ratio of A_1/A_2 selectivity could be altered depending on what molecule was attached to the functionalized xanthine congener. Thus, the congener approach may be expected to yield xanthines which are more selective in their physiological action.

A radioiodinated analog of theophylline is desired for studies on the adenosine receptor in tissues where it occurs in low levels. By attachment of the functionalized congener to a molecule which is subject to facile iodination, such as a phenol, this may be achieved. A substituted phenol was attached to an amino congener of 1,3-dipropyl xanthine resulting in binding affinity for the A receptor in the nanomolar range. Iodination studies are now underway. [K. Jacobson, K. Kirk, W. Padgett (LBC), J. Daly (LBC)]

The preliminary success towards the radioiodination of xanthines suggests a general approach to the design of radiolabelled drug analogs based as the congener approach. A functionalized drug congener, 4, may be attached to a molecule specifically designed to accept a particular radioisotope. By treating separately the receptor recognition moiety contained in the congener, 4, and the chemistry of the radio-isotope acceptor unit, 5, one has more freedom to design schemes for efficient reactions with radioisotopes.



When this scheme is applied to cyclotron-produced position emitters, such as ^{11}C and ^{18}F, the resulting drug conjugate 7 may be useful for tomographic studies. In PET scanning the efficiency and rapidity of the radiolabeling reaction are crucial variables and can be approached in a basic and general manner by studies of chemical models for the radioisotope acceptor unit. For example, for ^{18}fluorine PET studies, 5 may consist of a functionalized carbon triflate ester, in which the triflate would be displaced by ^{18}fluorine-fluoride ion. [K. Jacobson, K, Kirk, M. Channing (CC NM), W. Eckelman (CC NM)]

The autoradiographic localization of adenosine receptors in the brain has been reported (Paul Marangos, NIMH, S. Synder, Johns Hopkins University, and coworkers) and it would be desirable to visualize the receptor to a greater resolution. Fluorescent histochemical probes based on functionalized congeners of adenosine agonists and antagonists are being synthesized. A fluorescent dye, such as rhodamine may be bound to the drug covalently or through a tight protein-ligand association. A biotinylated conjugate of the adenosine amino congener (2) was synthesized and found to have an IC$_{50}$ of 12 nM in CHA displacement studies using rat cerebral cortex. This conjugate is expected to bind tightly to both the A$_1$-receptor and the glycoprotein avidin, derivatives of which may then serve as fluorescent or EM probes for the receptor. [K. Jacobson, K. Kirk, P. Marangos (NIMH), D. Jacobowitz, (NIMH)]

Recently it has been possible to isolate receptors for neurotransmitters from solubilized membrane preparations using affinity chromatography. Either an analog of the neurotransmitter or an antibody to the receptor is immoblized on an insoluble resin support. A column of such resin binds the receptor specifically but in a reversible manner. We have immobilized functionalized congeners of adenosine and its antagonists to sepharose resins and are now attempting to concentrate or isolate the receptor protein(s) chromatographically. [K. Jacobson, K. Kirk, P. Marangos(NIMH)]

For PET radiographic studies of the brain, it is necessary that the position-emitting drug analog enter the brain after iv. administration. Low brain uptake is also a general problem in the therapeutic administration of certain pharmaceutical agents. Particularly hydrophilic compounds which are not taken up by specific transport mechanism are excluded at the blood-brain barrier. Thus it is desirable to develop means to modify drugs to cross into the brain readily.

One method is the pro-drug approach in which hydrophilic or charged groups on the drug are covalently blocked in a reversible manner. After the drug has entered the brain by virtue of added hydrophobicity it is cleaved, thus regenerating the parent, biologically-active drug. An alternate approach is to design drug conjugates which are taken into the brain and do not require subsequent cleavage for biological activity. It is the latter, "new-drug" approach to which the functionalized drug congeners may be applied.

The goal is to select specific uptake systems of the blood-brain barrier (such as those for amino acids or carbohydrates) which may also be used to transport drug conjugates. For example, L-phenylalanine nitrogen mustards are cytotoxic agents which are carried into the brain by a transport system which recognizes the α-amino acid moiety. In our study, ^{14}C-labeled amino acid analogs are being measured for brain uptake in rats, to establish structure activity relationships of potential carriers moieties to which functionalized drugs may be attached. [K. Jacobson, K. Kirk, R. Blasberg (NCI)]

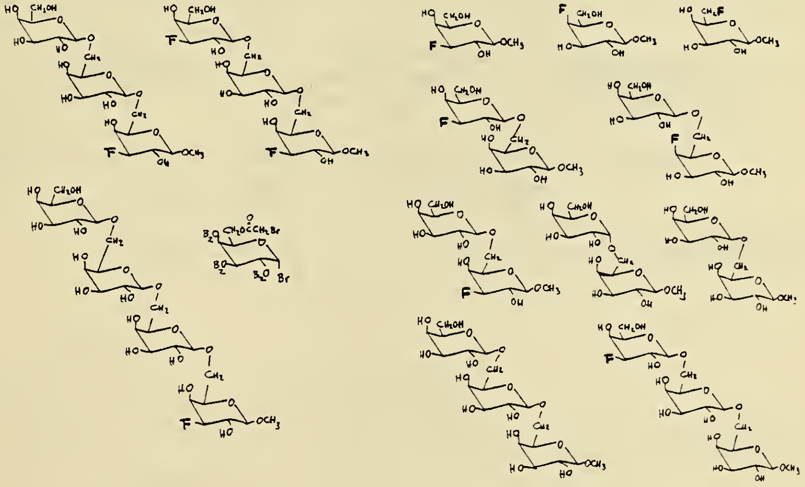
SECTION ON CARBOHYDRATES

BINDING STUDIES ON MONOCLONAL IMMUNOGLOBULINS:

A number of deoxy fluorogalactose derivatives (see below) have been prepared. The substitution of a hydroxy group in a ligand by a fluorine would be revealing if that hydroxy group would be required for hydrogen bonding of that ligand to a protein (in this case a monoclonal antibody with specificity for a polygalactopyranan. It was found that the 2- and 3- hydroxy groups of galactopyranose were required for binding of that ligand to the highest binding subsite of monoclonal IgA J539. By the measurement of the affinities of a series of oligosaccharide containing 3-deoxy-3-fluorogalactopyranose as part of its sequence, it has become possible to map the individual four subsites in the antibody capable of harboring the tetra saccharide sequence of the polysaccharide antigen. If the four subsites are named CABD starting near the 2nd hypervariable region of the H-chain of the protein and stretching across the antibody combining region towards the first hypervariable region of the L-chain, then their affinity decreases in the order ABCD. This is the first time antibody-antigen interactions were mapped with this degree of detail.

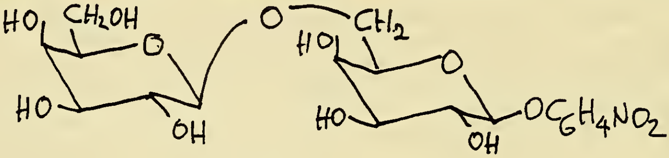
PREPARATION OF DEOXY-FLUORO-GALACTOSE DERIVATIVES:

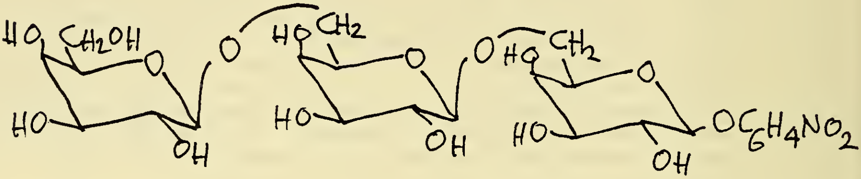
The following saccharides (next page) were synthetically prepared (final products are listed only, intermediates are not listed) as part of a very large synthetic program to sustain work on ligand/protein interactions (see also below) and hydrogen-bond studies.

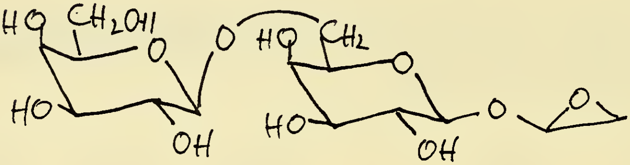


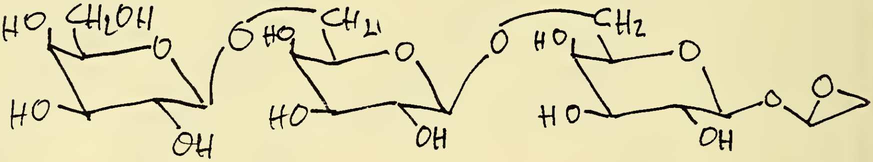
PREPARATION OF AFFINITY LABELS FOR GALACTAN-BINDING MONOCLONAL IMMUNOGLOBULINS:

In order to further study the location of the combining region of monoclonal antibodies with β(1→6)-D-galactopyranan specificity we have prepared a number of new affinity labels including:



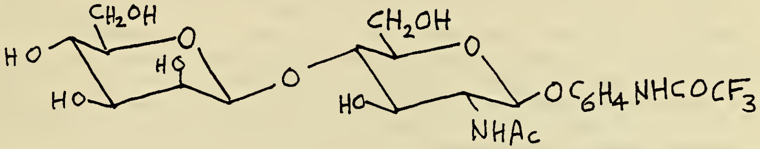


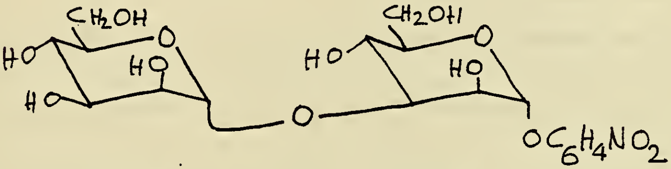


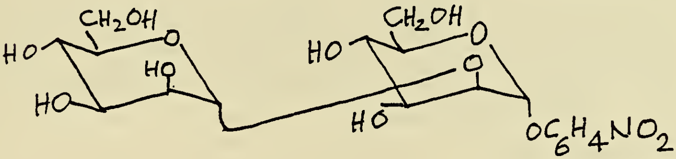


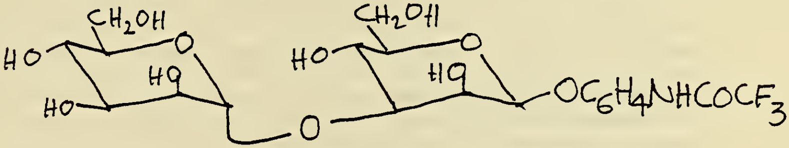
PREPARATION OF IMMUNODETERMINANTS KNOWN TO OCCUR ON GLYCOPROTEINS:

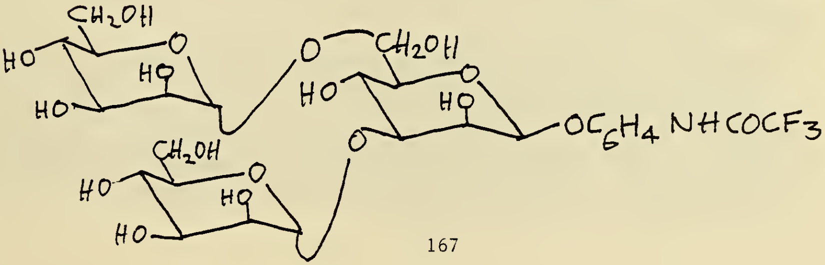
In addition to the oligosaccharidic determinants reported last year we have prepared the following (hitherto not synthesized) immunodeterminants listed below. These were then coupled to proteins and the resulting immunogens were then used to obtain diagnostic antisera to be used to screen cell surfaces involved in transformations. These sera are presently being immunocharacterized.











COLCHINOIDS:

Systematic studies with colchicine, exploring whether this alkaloid could by chemical manipulation be converted into a less toxic compound with potential use in gout and as an antitumor agent have continued. Affinity labeling of colchicine to afford compounds useful to map the colchicine binding site on tubulin, including spin-labeled and uv-sensitive compounds has been carried out, but did so far not give any useful information. Modification of colchicine which afforded potent compounds have been extended to thiocolchicine, a synthetic analog of colchicine chemically easier to manipulate. Several thiocolchicine analogs, highly potent in the tubulin binding assay and in the P388 assay in vivo have been obtained. Some are being evaluated at the NCI as potential antitumor agents, particularly 3-demethylthiocolchicine, which was found potent in several antitumor assays. These findings are now being extended to colchicides, colchinoids lacking the 10-OMe group, and to allocolchicines, compounds having an aromatic ring C instead of a tropolonic ring, and ultimately to biphenyls with hindered rotation. (Sharma, Kerekes, Brossi).

PERHYDROHISTRIONICOTOXINS:

These compounds are derived from natural histrionicotoxin and have biological activities involving the acetylcholine receptor and are of potential interest in connection with Alzheimers diseases, Myestenia gravis, nerve gas poisoning and disorders involving neuromuscular transmission. It was shown that the alkyl groups in HPTX were less important for biological potency in the frog muscle assay than the stereochemistry of the basic spiroamine moiety. In order to confirm this finding spiroamines without alkyl groups have been prepared. C-Alkylation of the naked keto-spirolactam with butyl bromide afforded after reduction as a major product the Kishi lactam prepared differently, suggesting that the "naked" ketolactam might be a potentially useful intermediate for the synthesis of histrionicotoxin itself. (Gessner, Brossi).

(+)-KETOMORPHINANS

Structure-activity-relationships in the (-)-series of morphinan analgetics is rather well established. It has now been substantiated that similar relationship relating (+)-morphinans with antitussive activity does not exist. It also is shown that the present tests to evaluate antitussive activity are unspecific and not very meaningful, and that new tests should be developed which are based on a better knowledge of antitussive action and possible receptor sites. (Kerekes, Chen, Brossi).

NEUROTOXIN MPTP:

MPTP, a man made neurotoxin is chemically related to some piperidine analgetics, and deserves also for this reason attention. Analogs of MPTP, possibly representing active metabolites have been prepared. The hypothesis that MPTP is further metabolized via its N-oxide, and that the latter may undergo conversion into dihydroderivatives and fully aromatic N-methylpyridinium salts is presently being checked. It is speculated that MPTP after oxidation to MPDP catalyzes the conversion of dopamine into 6-hydroxydopamine. (Gessner, Brossi).

TETRAHYDROISOQUINOLINES AND DOPAMINERGIC AND ADRENERGIC RECEPTORS:

All optically active isoquinolines so far tested and found active in screenings affecting dopaminergic and adrenergic receptors have been found to act stereospecifically, and of the two optical isomers only the S-isomers were found potent, the R-isomer leading in the series of reticulines to opium alkaloids were much less potent. Two racemic tetrahydroisoquinolines, one with an o-hydroxy and the other with a m-hydroxy group in the benzyl part showed reasonable potencies in the adrenergic screening and will be prepared in optically active forms. (Rice, Kerekes, Brossi).

ANTIMALARIAL AGENTS:

Qinghaosu, a Chinese antimalarial sesquiterpene peroxide is interesting because it is active against P. falciparum malaria resistent to antifolates and chloroquine. The metabolism of qinghaosu is being studies in man at several places but methods and means to prepared labeled materials are lacking. Two metabolites of qinghaosu in man could be derivatized as esters with diacetyldihydrofluoresceine (DADF) made visible on tlc-plates as red spots after treatment with ammonia and iodine vapors. Artesunate, a derivative of dihydroqinghaosu, and useful in cerebral malaria, is presently being studied and its potential as an antimalaria drug evaluated. (Luo, Brossi).

CHARACTERIZATION OF OPIATE RECEPTORS USING POSITRON EMISION TRANSAXIAL TOMOGRAPHY (PETT):

PETT scanning is a unique relativily non-invasive technique for visualization of drug receptors in the living human brain. In our recently initiated line of investigation for study of the structure and function of opiate receptors using PETT scanning, ^{18}F ($T_{1/2}$=110 min) was chosen as the position emitting isotope. Unlabeled fluorinated agonists (FOXY) and antagonist (CYCLOFOXY) drugs based on oxymorphone and naltrexone, respectively, have been synthesized. *In vitro* experiments have confirmed the suitability of these probes and preliminary experiments indicate successful synthesis of these compounds labeled with [^{18}F]. (K. C. Rice, T. R. Burke, Jr., S. Larson, R. Eckelman, M. Channing).

TOPOLOGICAL CHARACTERIZATION OF OPIOID RECEPTORS:

A second line of investigation relating opioid receptor structure and functions involves synthesis and biologically study of conformationally restricted 5-(m-hydroxyphenyl)morphans as topological probes for opioid receptor subpopulations. We are utilizing an oxide bridge to alter the orientation of the m-hydroxyphenyl substituent in increments of 60° relative to atoms 2,3,5,6 of piperidine ring in the rigid, parent ring system which has a freely rotating m-hydroxyphenyl group that can assume any orientation at the receptor. We have now synthesized 3 of the six possible isomers. Two are inactive as agonists or antagonists *in vivo* and do not bind appreciably to opioid receptors from rat brain. A third isomer behaves as a weak antagonist *in vivo* and binds moderately well (EC_{50} < 100 nm) to opiate receptors. Work is in progress to prepare the remaining three isomers. (K. C. Rice, A. E. Jacobson, T. R. Burke, Jr.).

CHARACTERIZATION OF OPIOID RECEPTORS USING IRREVERSIBLE INHIBITORS:

In a third line of investigation in our program to study structure and function of the opioid receptor-endorphin system, we have prepared a number of irreversible inhibitors of opioid receptors. In this group, compounds have been identified which are specific for the mu and delta receptor subpopulations. A mu specific and delta specific ligand have been tritiated to high specific activity. The first enantiomeric pair of alkylating opioid ligands have been prepared and show substantial stereoselectivity. Isolation and purification of opioid receptors is in progress (K.C. Rice, A. E. Jacobson, T. Burke, R. Lessor, W. Klee, R. A. Streaty).

AGONISTS AND ANTAGONISTS FOR THE PHENCYCLIDINE (PCP) RECEPTOR:

An electrophilic, irreversible inhibitor specific for phencyclidine receptors *in vitro* has been synthesized and evaluated *in vivo*: ICV administration of this compound has been shown to block the *in vivo* effects of PCP 24 hours after administration. *Ex vivo* experiments 24 hours after administration, and Scatchard analysis of functional receptors remaining confirmed the expected reduction in Bmax. (A. E. Jacobson, K. C. Rice, R. Lessor, T. O'Donohue, P. Contreras).

EVALUATION OF THE DEPENDENCE POTENTIAL AND ABUSE LIABILITY OF ANTINOCICEPTIVES

About 100 compounds, obtained form our own Laboratory and from universities and industrial groups in the US, Europe and Asia, were examined to determine their antinociceptive activity in various rodent assays. In collaboration with the Departments of Pharmacology of the University of Michigan Medical School and the Medical College of Virginia, under the auspices of the Committee on Problems of Drug Dependence, some of these compounds were further examined to determine their dependence potential and abuse liability. Data obtained from these studies has served to alert individuals about possible side-effects of compounds before clinical introduction of the drug, and to focus attention on those drugs which might serve as specific probes for subpopulations of opiate receptors. (A. E. Jacobson, E. L. Atwell, J. H. Woods, J. L. Katz, F. Medzihradsky, C. B. Smith, L. S. Harris, M. D. Aceto, E. L. May, and G. D. Winger).

TOTAL SYNTHESIS OF OPIOIDS VIA DIHYDROTHEBAINONE AND DERIVATIVES.

The NIH Opiate Total Synthesis in its present form now permits practical total synthesis of either the natural or unnatural enantiomers of morphine codeine and thebaine in any desired quantity with about 25% overall yield from m-methoxyphenethylamine, a commercially available starting material. Since these three alkaloids are the only raw material utilized for production of all opium-derived narcotics and their antagonists, the NIH synthesis renders the entire spectrum of these medically essential drugs available by total synthesis. The synthetic route has now been modified to provide a substantially shorter thebaine-free total synthesis of the agonist-antagonists buprenorphine and nalbuphine and the narcotic antagonist naltrexone and nalmefene. The total synthesis may became essential for production of sufficient amounts of the narcotic antagonists should they find medical utility in the treatment of obesity, Alzheimers disease, or cardiovascular shock. (K. C. Rice).

ISOLATION AND CHARACTERIZATION OF THE ANTITRYPANOSOMAL FACTOR FROM *P. FLUORESENS*.

Major components of the organic soluble extract of *P. fluoresens* have been isolated and identified as long chain fatty acids which are toxic to *T. cruzi* but are also hemolytic. Biological studies of the effects of other compounds against *T. cruzi* are planned (K. C. Rice, T. Burke, T. Mercado).

SYNTHESIS AND EVALUATION OF POTENTIAL CNS, ANTIINFLAMMATORY AND ANTICANCER AGENTS.

Stereoselectivity of the enantiomers of (+)- and (-)-PCMP, (a closely related derivative of phencyclidine) was found in several *in vivo* systems in which the (+)-enantiomer was phencyclidine-like while the (-)-enantiomer was essentially inactive. This is consistent with eariler findings that the (+)-enantiomer was more active in a number of systems used to detect phencyclidine activity. An alkylating [^{3}H]phenothiazine was synthesized and utilized for formation of a covalent complex with calmodulin, which was further characterized as having at least two phenothiazine binding sites. (K. C. Rice, T. R. Burke, C. Klee, T. R. Burke, Jr., D. Newton).

CHARACTERIZATION OF OPIOID RECEPTORS USING RIGID PROBES.

Irreversible ligands from the endoethenooripavine family of opioids have been synthesized which can covalently interact with either mu or delta subpopulations of opioid receptors. The interaction allows a view of the three-dimensional spatial positioning of the alkylating moiety necessary for differentiating between its reaction with the mu or delta opioid receptor populations, because of the rigidity of the endoethenooripavine nucleus. These data could lead to placement in three-dimensional space of those amino acids bearing amino or sulfhydryl groups, in computerized models of the interacting receptor matrix and ligand. (A.E. JAcobson, K. C. Rice, R. A. Lessor, W. A. Klee).

SECTION ON METABOLITES

INTERACTION OF ANALOGS OF HISTRIONICOTOXIN WITH THE ACETYLCHOLINE RECEPTOR IONIC CHANNEL COMPLEX AND MEMBRANE EXCITABILITY:

The effects of the four N-benzylazaspiro analogs of histrionicotoxin, which are without the two side-chains typical of histrionicotoxin, were studied on the ionic channels of electrically excitable membrane and the nicotinic acetylcholine receptors in frog sartorius muscles. Each analog reversibly blocked the indirectly elicited twitch and potentiated the directly elicited twitch in a concentration-dependent manner. The analogs decreased the amplitude and rate of rise and prolonged the falling phase of the directly elicited action potential and blocked delayed rectification suggesting blockade of sodium and potassium conductances. All of the analogs caused a concentration- and voltage-dependent depression of the peak end-plate current amplitude and induced nonlinearity but no hysteresis or time dependency in the current-voltage relationship. The marked shortening of the time constant of end-plate current decay produced by the analogs was concentration-dependent. The relationship between the time constant of end-plate current decay vs. membrane potential remained a single exponential function of time despite the marked shortening of the decay phase and loss of voltage dependence. The effect of the analogs on miniature end-plate current was identical to that on end-plate current. Single channel conductance was unaffected by the analogs, but the single channel lifetime was shortened. The marked shortening of the time constant of the end-plate current decay and single channel lifetime plus linear relationship between reciprocal of the time constant of decay vs. analog concentrations strongly suggest that the analogs interact with the ionic channels of the nicotinic acetylcholine receptor in their open conformation. The interaction of these analogs with the ionic channel of the acetylcholine receptor in open conformation are in marked contrast with that of histrionicotoxin. Finally, the removal of side-chains from the analogs restricted their interaction to only one type of conformational state (M. A. Maleque, A.Brossi, B. Witkop, S. A. Godleski and E. X. Albuquerque).

A Study of the Novel Synthetic Analog (+)-Depentylperhydrohistironicotoxin on the Nicotinic Receptor-Ion Channel: (±)-Depentylperhydrohistrionicotoxin [(±)-Depentyl-H$_{12}$-HTX) lacks the 5-carbon side chain. Recent studies with N-benzylazaspiro analogs of histrionicotoxin (HTX) in which both side chains are removed revealed that this alternation restricted the action of the compounds on the acetylcholine receptor-ionic channel complex (AChR) to an open channel blockade. Thus, an important question was raised as to the role of the side chains in affecting the interaction of these inhibitors with the AChR. In addition, the effect of (±)-depentyl-H$_{12}$-HTX on membrane excitability was investigated. (±)-Depentyl-H$_{12}$-HTX blocked the indirectly elicited twitch. It decreased the emplitude and rate of rise and prolonged the falling phase of the action potential and blocked delayed rectification suggestive of blockade of sodium and potassium conductances. However, its effects on sodium and potassium conductances were less marked than those of HTX. It decreased the peak amplitude of the end-plate currents (EPCs) and accelerated the decay time constant of EPCs (τ_{EPC}) currents in a concentration-dependent nonlinearilty in the current-voltage relationship of EPCs. Despite marked shortening of τ_{EPC}, the decay phase of the EPC remained a single exponential function of time. Single channel conductance was unaffected by the analog, but the single channel lifetime was shortened. The voltage- and time-dependent effects of the analog that occurred without prior activation of AChR suggest reaction with the ionic channel in its closed conformation. However, marked shortening of τ_{EPC} and single channel lifetime, in addition to the linear relationship between $1/\tau$ and (±)-depentyl-H_{12}-HTX concentration, strongly suggest that the analog reacts with the ionic channel in its open conformation. In this respect effects of (±)-depentyl-H_{12}-HTX are different from those of HTX or the N-genzylazaspiro analogs. The main differences appear to be related to chemical structure such that the two side chains allow HTX or H_{12}-HTX to interact with the ionic channel predominantly in its closed conformation, whereas the core of the molecule lacking side chains in the form of the N-benzylazaspiro analogs allows strong interaction with the ionic channel in its open conformation. (±)-Depentyl-H_{12}-HTX having one side chain represents an intermediate compound which reacts with both closed and open conformations but primarily the open state of AChR (M. A. Maleque, K. Takahashi, B. Witkop, A. Brossi & E. X. Albuquerque).

Modeling Studies on AchR Agonists: The specific structures are: (+)anatoxin-a (unprotonated and protonated); methyl (protonated) and Dimethyl anatoxin-a, nor-ferruginine (unprotonated and protonated); Ferruginine (protonated), Ferruginine methiodide and isoanatoxin-a (unprotonated and protonated).

The compounds were first modeled by means of the Merck Molecular Modeling (MMMS). The structures were then fully energy-geometry optimized using Allinger's Molecular Mechanics (MM2) Program. Prior to modeling, the latter program was altered to handle charges. Structural parameters for ammonium slats were generated from x-ray diffraction data on methyl, polymethyl, penta- and hexacyclic ammonium slats. Charge parameters were derived from 6-31G* *ab initio* calculations on several of the salts. Results obtained from MM2 using these parameters agree very well with the *ab initio* and MNDO calculations for charge densities and relative energies. Steric energies; C=C-C=- dihedral angles for the s-trans and s-cis isomers and the non-bonded distances between the carbonyl oxygen at its Vander-Waals extension and the nitrogen were calculated and correlated. For the unprotonated and protonated anatoxin-a, only the boat conformations were observed for the seven-membered ring, and two twisted conformations for the five-membered ring. The difference in energy between the latter two is estimated to be about 1.0 kcal. For the in protonated and protonated anatoxin-a, the s-trans conformer is lower in energy than the s-cis by about 0.5 kcal. For the protonated anatoxin-a, the difference between the s-trans and the s-cis isomers is larger by about 8.0 kcal. This calculation was checked with MNDO with the found results. We are not yet sure what meaning,if any, should be ascribed to the dihedral angles for the s-trans and s-cis. Nonetheless, the measured non-bonded distances between the nitrogen and Vander Waals radius of the carbonyl oxygen indicate that the s-cis isomers have the proper Beer's and Reich distance, whereas for the s-trans isomers this distance is too short. The lowest energy conformer for protonated methyl and dimethyl anatoxin-a is the chair (PMANA12A and DMANA01). However, in both cases, the nitrogen to carbonyl oxygen distance is very short (@ 4.1A°). Again, only the s-cis conformers attain the Beers and Reich distance. We took the s-trans-chair conformer (PMANA12A) and rotated the C_2-C_3 single bond in $c=c_2-c_3=0$ from 180 to 0°. The energy of the s-cis conformer was bout 9 kcal higher and the chair was still present though there is no calculated barrier between the two rotamers. For the dimethyl anatoxin-a, the s-cis isomer exists only in the boat. We have performed a calculation varying the dihedral angle around the carbonyl and found that at angles between -066° the boat prevailed (Energies = 31→29 kcal) while at 96° the chair conformer formed (Energy = 17.4 kcal) and achieved its lowest energy at 156° (Energy - 12.1 kcal). The unprotonated and protonated nor-ferruginines, ferruginines and ferruginine methiodides show similar results, except that the six-membered ring is relatively flat. Again the s-trans isomers are of lower energy than the s-cis by about 8 kcal, and the Beers and Reich distance can only be reached by the S-cis. The isoanatoxin series shows the chair to be of lower energy than the twist boat conformers for the unprotonated case, and the s-trans .5 kcal lower in energy than the s-cis. The Beers and Reich distance is obtained for the s-cis and it doesn't matter if the second six membered ring is chair or twist boat. For the protonated isoanatoxin-a only the chair is obtained, s-trans below s-cis by 8 kcal, and the Beers and Reich distance can only be obtained by the s-cis. These results in general predict that if the distance criterion is most important, then all of these should bind to the receptor.

These model studies, done by Dr.Tamara Gund, New Jersey Institute of Technology, Newark, N. J., are now considered important enough to be supported by the US Army with a grant approaching $0.5 million.

Optical Antipodes of Agonists (+) and (-)-Anatoxin a: The synthesis of antipodes of anatoxin a, originally projected to be done within the Laboratory of Chemistry, by Drs.A. Brossi and Dr. Gessner, has now been accomplished at University of California, Berkeley by arrangement with Prof. Henry Rapoport, starting with D- and L-glutamic acid, respectively. These antipodes are now under electrophysiological investigation in the laboratory of Professor Edson X. Albuquerque.

Neurochemical Aspects of Alzheimer's Disease: A collaborative program with Dr. Stanley Rapport, Nat. Inst. of Aging, has been initiated to screen agonists for candidates in connection with Alzheimer's disease, the largest single cause of loss of memory function in the elderly population of the Western world, in most cases a rather specific disorder of cholinergic neurons. It should be possible to develop effective treatments for the symptoms of this disease. It is only recently that Alzheimer's disease has come to the attention of the majority of the public and scientific communities. One reason for this sudden rise to fame has been the recognition that at least half of the elderly patients said to be suffering from 'senility'; 'organic brain syndrome' or 'hardening of the arteries' actually have in the brain the pathologic signs of Alzheimer's disease, without any other significant brain disease. The direct examination of brain tissue to reveal the presence of these pathological features, the neurofibrillary tangle and the neuritic plaque, remains the only way to definitively diagnose this disease. The best estimates of the size of this patient group suggest that there are about 1.5 million such individuals in the USA alone. Hence the recent attention given to Alzheimer's disease seems both justified and long overdue. Attempts to enhance central cholinergic transmission must be selective: non-selective enhancement of acetylcholine release could clearly result in enhanced transmission in pyramidal and extrapyramidal motor systems, probably producing undesirable side-effects. What is required is the ability to enhance cholinergic transmission in the nucleus basalis-cortical system without disruption of normal transmission at other cholinergic synapses. One high proprity area for research might be to search for agents which were muscarinic agonists selective for receptors in cortical and hippocampal regions. Another approach might be to selectively enhance the sensitivity of muscarinic acetylcholine receptors on cortical and hippocampal neurons. Other possibilities include a search for receptors activating cholinergic neurons ofthe nucleus basalis, to attempt to drive these cells to release more acetylcholine into the deficient regions. Methods to enhance acetylcholine synthesis at terminals within the cortex and hippocampus may also exist; it would appear that only a small fraction of the choline acetyltransferase in synaptic terminals is active in acetylcholine synthesis, although the reasons for this are far from clear. Another possible therapeutic avenue is the only one that has so far produced reproducible improvements in patients in double blind studies. Patients have been treated with oral physostigmine, a naturally occurring cholinesterase inhibitor (usually incombination with lecithin) or synthetic cholinesterase inhibitors, such as prostigmine or pyridostigmine. In this respect completely unexpected effects, found for these inhibitors, in the Laboratory of Professor Edson X. Albuquerque, have to be taken into consideration.

When the actions of pyridostigmine (Pyr), an anticholinesterase agent, were studied on the acethylcholine (ACh) receptor-ion channel complex and on the electrically excitable membrane of the frog cutaneous pectoris and sartorius muscles and the chronically denervated soleus muscle of the rat, Pyr at concentrations of 0.2-0.4 mM potentiated the indirect evoked muscle twitch and at concentrations $\geq$ 0.8 mM depressed the indirect twitch with an IC_{50} of about 2 mM. Twitch depression produced by Pyr was reversed slowly, and after a 60-min wash only 59% of the control muscle twitch had returned. Pyr did not affect either the membrane potential or the muscle action potential. Pyr had several effects at the neuromuscular junction of the frog and rat. It decreased the peak amplitude of the end-plate current (EPC) in a voltage- and concentration-dependent manner. In contrast to diisopropylfluorophosphate, which depresses the EPC amplitude and induces a double exponential decay of the EPC and miniature end-plate current (MEPC), Pyr produced a marked prolongation of the time constants of EPC and MEPC decay while maintaining a single exponential decay. The decrease caused by Pyr of indirect twitch tension, ePC amplitude, and ACh sensitivity indicates mechanisms which limit the number and/or properties of conducting channels. The drug decreased channel conductance and prolonged channel lifetime as revealed by Fourier analysis of ACh-induced end-plate current fluctuations. An altered form of the conducting species induced by Pyr appears to be responsible for either the apparent agonist-induced depolarization or its ability to increase the affinity of ACh for its recognition site. Pyr was also found to inhibit the binding of ACh and α-bungarotoxin to receptor-rich membrane from the electric organi of Torpedo nobiliana, and to have a higher affinity for the receptor than for the ion channel binding sites. These actions are distinct from acetylcholinesterase inhibition caused by the agent. Strong evidence suggets that the direct influences of the agent on neuromuscular transmission involve at least three distinct, although possibly interacting, mechanisms: (a) a weak agonist action, (b) the formation of desensitized receptor-complex intermediates, and (c) the alteration of the conductance properties of active channels.

As this contribution from the Labortory of E. X. Albuquerque reveals, both physostigmine and pyridostigmine act as weak agonists with the acetylcholine receptor-ionic channel complex and are capable of inducing desensitization. This phenomenon of desensitization may probably be responsible for numerous cases of death observed after administration of high doses of physostigmine or prostigmine to patients suffering from myasthenia gravis.

This new aspect of the mechanism of action of anticholinesterase agents with the AcChR, as it will become known to a wider public, will undoubtedly rekindle interest in new and old derivatives of physostigmin and its analogs and congeners. For this reason congeners of physostigmin, such as physovenine and geneserine, are to be included in this program (collaborating unit: Prof. Brian Robinson, Department of Pharmacy, The University, Manchester, England).

From Muscarinic to Nicotinic Agonists as a Function of Charge and Rigidity: Arecolone methiodide is 8.6 times more potent than carbamoyl-choline (standard nicotinic agonist). The non-quaternized arecolone was inactive. Arecoline methiodid (a 3-carboxymethoxy ester replaces the 3-acetyl group of arecolone) was moderately active (rel. pot. = 1.3). 4-Methylarecolone and 1-methyl-3-(2-hydroxyethyl)-1,2,5,6-tetrahydropyridine methiodides had rel. pot. of 0.05 and 0.01, respectively. (-)-Ferruginine methiodide displayed a potency of 3.3. Interestingly, (-)-nor-ferruginine displayed 1/300 the activity of its homolog (+)-anatoxin-a (J. A. Waters, Ch. Spivak, E. X. Albuquerque).

Search for New Membrane-Active Compounds: Synthesis of Bridgehead Alkylated Tropanols: The synthesis of bridgehead alkylated tropanols 1 as models for histrionicotoxin has been developed. The synthesis of 1 started from furfuryl alcohol via a Robinson condensation to 2. Conversion of 2 into the key 1-aldehyde allows for variations in the structure and length of the side chain. So far 1 and its dihydro derivative have been prepared. En-yne types that are structurally closer to histrionicotoxin shall be the next target. Biological screening is in progress at the Department of Pharmacology and Experimental Therapeutics, University of Maryland Medical School (G. Fodor and R. Dharanipragada, Department of Chemistry, West Virginia University, Morgantown, West Virginia).

Birth and Rebirth of Leading Ideas: A lecture under this title was delivered on the invitation of the Medical Faculty of the University of Frankfurt. Progress in science depends on progress in methods. More than 2000 Protein sequences were elucidated with the help of two chemical cleavage methods, the Edman degradation and the cyanogen bromide cleavage. What is less well known is the logical and historical connection that exists between the timing and origin of the cyanogen bromide cleavage and the development of the first gel-based radioimmunoassay techniques by Jerker Porath and Rolf Axen. As Prof. Porath described this sequence of events in his own words:

> "Axen joined Bernhard Witkop at Bethesda to learn new techniques for selective cleavage of protein. Upon his return to the Institute of Biochemistry at Uppsala, he suggested a new approach to our project, viz., the coupling of substances containing primary amino groups to cyanamide-Sephadex. The idea was suggested as a research project to Sverker Ernback, one of our first-year research students. The cyanamide-Sephadex was prepared by treating amino-Sephadex with cyanogen halide. To my surprise, the yield of coupled amino acid exceeded 100

> percent! I urged Ernback to make blind experiments using unsubstituted Sephadex. Indeed, Sephadex after cyanogen halide treatment was found to immobilize protein in excellent yields!
>
> "Cyanogen bromide coupling replaced the isothiocyanate procedure for the synthesis of immobilized antigens and antibodies to be ued in radioimmunoassays. It is still the most commonly used method for preparating gel- and paper-based immunodiagnostics (RIA, RAST, PRIST, etc.).
>
> "Our discovery of the cyanogen halid coupling method was followed by hectic work in several directions. Enzymes and enzyme inhibitors were immobilized onto a variety of hydroxylic supports. Not much later, our work on activation of agarose for enzyme immobilization was described, and this work initiated an almost explosive development in (bio-)affinity chromatography.
>
> "We interpreted the activation to involve the formation of cyanate followed by its rapid conversion to imino carbonate. The final coupling products were thought to be gels containing mixtures of imino carbonic acid esters, carbonic acid eters, and carbamate substituents, and, somewhat later, isourea linkages were also considered. Evidence for this interpretation was obtained from IR-spectra including also some model compounds."

M. Wilchek, like Axen a Visiting Fellow in the Laboratory of Chemistry, determined the active species in cyanogen bromide-activated polysaccharides.

> "The complicated scheme of reactions is now fairly well understood, thanks to Meir Wilchek and others. The nucleophilic displacement of the ligands with ammonia is particularly interesting: original amino groups are converted into guanidino groups. By using the cyanogen bromide activated support as an organic reagent, Wilchek converted insulin into 'superinsulin'.
>
> "Our original suggestions have been essentially confirmed, but the recent work has shed light on some important limitations. In improved form, the cyanogen bromide coupling is still the preferred method for the preparation of most biospecific adsorbents used in (bio)affinity achromatography, and agarose is by far the most commonly employed support." [Cf. Current Contents, This week's Citation Classic, May 28, 1984, Number 22, p. 21].

CHEMICAL SYNTHESIS AND BIOLOGICAL ACTIVITY OF SEQUENCE-SPECIFIC 2-5A ANALOGS:

A rapid chemical approach to the preparation of sequence-specific 2',5'-oligonucleotides and analogs of 2-5A is described. For instance, reaction ofthe 5-phosphoromorpholidate of adenosine (MopA) with the 5'-phosphoroimidazolidate of inosine (ImpI) under conditions of lead ion catalysis, gave MopA2'p5'I in 21% yield. Acid hydrolysis of MopA2'p5'I gave pA2'p5'Iwhich then was converted to the corresponding 5'-phosphoroimidazolidate Imp5'A2'p5'I through redox condensation with triphenylphosphine, imidazole and 2,2-dipyridyldisulfide. Lead ion-catalyzed condensation of the above product with Mop5'A gave Mop5'A2'p5'A2'p5'I in 17% yield. By acid hydrolysis, the above trimer could be converted to the corresponding 5'-monophosphate, or by reaction with pyrophosphate in DMF, to the corresponding 2-5A analog, ppp5'-A2'p5'A2'p5'I. Using similar methodology, the following oligonucleotides were prepared: ppp5'A2'p5'A2'p5;A, ppp5'A2'p5'I2'p5'A, ppp5'I2'p5'A2'p5'A2'p5'A and ppp5'A2'p5'A2'p5'(2'dA).

Binding and activation efficiency to the 2-5A-dependent endonulcease by chemically synthesized partially inosine-substituted 2-5A analogs, namely, pppI2'p5'A2'p5'A, pppA2'p5'I2'p5'A and ppA2'p5'A2'p5'I were compared with that of native 2-5A (pppA2'p5'A2'p5'A)in the mouse L cell and human lymphoblastoid (Daudi) cell extracts. pppI2'p5'A2'p5'A was found to bind to the endonuclease 140 and 270 times less strongly than native 2-5A in Daudi and L cell extracts, respectively, whereas both pppA2'p5'I2'p5'A and pppA2'p5'A2'p5'I showed only a few times weaker binding affinity to the endonuclease compared to 2-5A in both extracts. On the other hand, when the protein synthesis inhibitory activity of these analogs were examined in L cell cell-free system, pppA2'p5'A2'p5'I showed no inhibitory activity until the concentration of 10^{-6}M and pppI2'p5'A2'p5'A and pppA2'p5'I2'p5'A showed 200 times and 20 times weaker inhibitory effects than native 2-5A (CI$_{50}$, 1.5 x 10^{-9}M). Futhermore, no 2-5A specific ribosomal RNA cleavage pattern was observed in the agarose gel electrophoresis when pppA2'p5'A2'p5'I (up to 10^{-4}M) was incubated with L cell or Daudi cell extracts, in which pppA2'p5'A2'p5'A, pppI2'p5'A2'p5'A and pppA2'p5'I2'p5'A showed distinct cleavages at concentrations of 10^{-9}, 10^{-7} and 10^{-8}M, respectively.

IN VIVO BIOLOGICAL ACTIVITY OF CORDYCEPIN ANALOGS OF 2-5A:

Analogs of the triphosphate 2'-5' linked adenylate trimer [ppp5'A2'p5'A2'p5'A, called 2-5A] which contain 3'-deoxyadenosine (cordycepin) instead of adenosine either in positions one and two, or in all three positions, are 10-100 fold less potent than is parent 2-5A in inhibition of protein synthesis in intact cells, when utilizing calcium coprecipitation techniques to introduce the 5'-triphosphate oligonucleotides into the cells. That the inhibition of protein synthesis was a consequence of activation of the 2-5A-dependent endonuclease by the 3-deoxyadenosine analogs of 2-5A was confirmed in obtaining the ribosomal RNA cleavage pattern that is characteristic of endonuclease activation by parent 2-5A. Additional results (i.e., lack of activity by the dimer species ppp5'(3'dA)2'p5'(3'dA) or the monomer 3'dA) as well as kinetic analysis both in intact cells and in cell-free extracts provided further evidence that the inhibition of protein synthesis observed with these 3-deoxyadenosine 2-5A analogs was not due to their degradation to the antimetabolite monomer unit 3'-deoxyadenosine.

SYNTHESIS AND BIOLOGICAL ACTIVITY OF 8-BROMO-ANALOGS OF 2-5A:

(2-5) 8-Bromoadenylate trimer 5'-monophosphate [2',5'(pbr^{8}A)$_{3}$] was synthesized by lead catalyzed polymerization of 8-bromoadenosine 5'-phosphorimidazolide according to Sawai *et al*. and converted into 5'-di-, and triphosphate. The activity of these compounds as activators of the 2-5A-dependent endonuclease (RNase L) was compared with the activity of (2-5) adenylate trimer 5'-triphosphate *in vivo*, in mouse L-cell extracts. All of the above analogs were activators of RNase L albeit less potent than 2-5A itself, 2',5'(pbr^{8}A)$_{3}$,2',5' (pbr^{8}A)$_{3}$ and 2-,5- pp(pbr^{8}A)$_{3}$ bound to crude RNase L preparations 30, 4 and 16 times less effectively than 2',5' pp(pA)$_{3}$, and inhibited protein synthesis to 50% at concentrations 250, 20 and 60 times higher than that of 2',5' pp(pA)$_3$. All compounds gave rRNA cleavage patterns characteristic of RNase L action. 2',5' (pbr^{8}A)$_3$ is the first known in vitro active analog of (2-5) oligo(A) with only one phosphate group at the 5'-end of the molecule.

ANTIMITOGENIC ACTIVITY OF 2'-5'-LINKED OLIGONUCLEOTIDES:

A large number of oligoadenylates have been investigated for their antimitogenic activity in synchronized (serum-starved) Balb/c 373 fibroblasts. Antimitogenic acitivity was determined in three different ways: (a) inhibition of [^{3}H]-thymidine incorporation, (b) inhibition of [^{35}S]-methionine incorporation, or (c) inhibition of cellular proliferation. By all criteria, the most effective antimitogenic oligoadenylate tested was the tetramer p5'A2'p5'A2'p5'A2'p5A. Other less effective agents were (in order of decreasing activity) p5'A2'p5'A2'p5'A > A2'p5'A2'p5'A > ppp5'A2'p5'A2'p5'A. Studies on the influence of chain length, 5'terminal phosphate modification and base or sugar alterations also have been examined. While the mechanism of action of these agents has not yet been established, the results obtained show that it is possible to use the chemical modification to potentiate the biological activity of 2-5A in intact cell systems.

POTENTIATION OF THE ANTIVIRAL ACTIVITY OF 2-5A. ANTIVIRAL ACTIVITY OF A CHEMICALLY STABLILIZED 2-5A ANALOG UPON MICROINJECTION:

2-5A [ppp(A2'p)$_n$5'A] has been implicated as a mediator in the antiviral action of interferon. Its direct evaluation as an indicator of virus replication is hampered by two limitations: its inability to penetrate intact cells, and its rapid intracellular degradation by (2'-5')-phosphodiesterase. These problems were overcome by using a microinjection technique whereby a phosphodiesterase-resistant analog of 2-5A, in which the 2'-terminal adenosine residue is replaced by 2-(9-adenyl)-6-hydroxy-methyl-4-hexylmorpholine, was injected into individual HeLa cells before infection with mengovirus. This microinjection of the hexylmorpholine 2-5A analog led to a marked reduction in virus yield, which depended on the dose of the 2-5A analog and the time (relative to virus infection) at which it was injected, suggesting a much higher stability than the natural 2-5A.

POSITIVE ION FAST ATOM BOMBARDMENT MASS SPECTROSCOPY OF-2-5A TRIMER CORE AND ITS 5'-MONOPHOSPHATE:

Mass spectrometry of 2-5A derived oligonucleotides has now been examined using fast action bombardment (FABMS). Specifically FABMS has been applied to 2-5A trimer core (1) and its 5'-monophosphate (2). The positive ion FAB mass spectra of the sodium salts of 1 and 2 show a series of molecular weight related ions of the form $(M + (1-x)H + Na_x)^+$, allowing unambiguous assignment of the molecular weight. The number of sodium cationized ions observed in the $(M + (1-x)H + Na_x)^+$ series is dependent on the number of phosphate hydroxyls and is equal to $P(OH)_x + 1$ when all labile protons have been exchanged with sodium.

Although the positive ion FAB mass spectra are complex due to the formation of various sodium and glycerol adducts, sequence information may be obtained in a manner apparently analogous to that used to sequence oligodeoxynucleotides using negative ion FABMS.[1] Fragmentation pathways leading to a number of significant ions in the mass spectra of 1 and 2 are proposed and the possible identity of these ions for the free compounds is illustrated. These results are based on comparisons with other related analogs, such as 2-5(7-deaza)A trimer monophosphate, which indicated similar fragments with appropriate mass shifts.

In conclusion, positive ion FABMS may be used to obtain molecular weight and sequence information on oligonucleotides. In particular, 2-5A and its synthetic oligonucleotide derivatives may be successfully characterized by this ionization method.

A HIGH-ACTIVE POLY(G)·POLY(C) INDUCER OF INTERFERON:

Experimental conditions necessary for the full expression of interferon-inducing activity by a complex of polyguanylic acid and polycytidylic acid included: (a) a sufficiently high molecular size of each homopolymer; (b) annealing conditions which insured complete denaturation of polyguanylic acid self-structure; and (c) the specific biological determine employed to assay interferon-inducing potency. The complex of polyguanylic acid and polycytidylic acid possessed several properties that suggested it may be an atypical polynucleotide interferon inducer. For instance, it was inactive in primary rabbit kidney cell cultures, usually exquisitely sensitive to polynucleotide interferon inducers, unless it was incubated on the cell cultures for prolonged times or in the presence of DEAE-dextran. Polyguanylic acid·polycytidylic acid could induce interferon in rabbits and mice but gave a more protracted response than did poly(I)·poly(C) and was, without any modification, resistant to degradation by serum nucleases.

SECTION ON MICROANALYTICAL SERVICES AND INSTRUMENTATION

SERVICE FUNCTIONS AND INSTRUMENTATION:

Basic research and service functions are performed by members of the Section. A major mission of the organization involves the instrumental and chemical analyses provided to scientists of the Laboratory of Chemistry, Laboratory of Bioorganic Chemistry, NIH and to a limited extent to personnel of other government agencies. Approximately 30 of the more common elements and five functional groups are determined on a quantitative basis, using ultramicro, micro, and semi-micro techniques as required. The materials analyzed included organic and inorganic research samples, commercial preparations and various biological specimens. Instrumental analyses include: GC/MS spectrometry, gas-liquid chromatography, GC with radioactive monitoring, unfrared, nuclear magnetic resonance, amino acid analysis, ultraviolet and flame photometry. Assistance in the interpretation of spectra is rendered on request. (D. F. Johnson, H. J. C. Yeh, N. Whittaker, W. White, P. Parisius, B. Torain).

NATURE OF STEROID-RECEPTOR INTERACTIONS

The objective of this project is to define the initial, intra-cellular events of steroid hormone action. Synthetic glucocorticoid derivatives, some of which react to form covalently labeled receptors via affinity labeling, have been prepared and are being used with glucocorticoid-responsive rat hepatoma tissue culture cells to examine: (1) steroid-receptor binding site interactions; (2) the effects of steroid binding on receptor conformation; and (3) the nature of "activation" of receptor-steroid complexes. We have combined affinity labeling by dexamethasone 21-mesylate with limited protease digestion in order to study various physical states (i.e., activated and inactivated) of covalent receptor-steroid complexes and to assess receptor-homology in different target tissues. These studies indicate that if activation of glucocorticoid receptor-steroid complexes is accompanied by changes in conformation or oligomerization (as has been suggested by others), the changes are not detectable by these methods and must be of limited magnitude. We have also synthesized a highly fluorescent glucocorticoid derivative (Dex-C_2-Rho). This steroid yielded the first specific, fluorescent glucocorticoid receptor-steroid complex, which should be very useful in the study of receptor-steroid complexes by fluorescence spectroscopy. (S. S. Simons, B. F. Torain, M. Pons, L. Mercier, T. E. J. Robinson, H. J. Eisen, E. I. B. Thompson).

APPLICATIONS OF NMR IN BIOCHEMICAL AND BIOLOGICAL SYSTEMS:

The metabolism of olefins and arenes in plants, animals and fungi appears generally to proceed via initially formed epoxide and arene oxide intermediates, which are frequently found in high optical yields. Earlier studies have been concerned with the determination of optical purity and absolute sterochemistry of epoxides and arene oxides using a wide range of methods. Although chiral shift reagents have been used to estimate the optical purity of individual chiral epoxides, these studies have generally been confined to epoxides derived from acyclic olefins and keto epoxides and have not been used in absolute stereochemistry determinations. An attempt was therefore made to obtain both optical yields and absolute stereochemistry of a wide range of chiral epoxides and arene oxides which have previously been resolved and configurationally assigned. The optical purities of these compounds were determined directly from anisochronous nmr signals for discrete enantiomers in the presence of chiral lanthanide shift reagents [$Eu(t.f.c.)_3$ and $Eu(h.f.c.)_3$]. The $Eu(t.f.c.)_3$ method is found to be useful for predicting the absolute sterochemistry of non-K-region and bay region tetrahydro epoxides and K-region arene oxides.

Qinghaosu (QHS), a sesquiterpene lactone with a peroxide bridge, first isolated in China from Artemisia annua L., and its analogs artemether and artesunate are clinically effective against P. vivax and chloroquine resistant falciparum malaria and cerebral malaria. These drugs represent a new type of antimalarial drug of considerable promise since their antimalarial effect in not antagonized by p-aminobenzoic acid. The absolute structures of several analogs derived from QHS were successfully elaborated by nmr spectroscopy. An α and β interconversion, presumably through an aldehyde-alcohol intermediate, was found to take place in solution of dihydroqinghaosu. The rate and equilibrium position of the α - β interconversion are solvent dependent.

Nmr spectroscopy of partially oriented molecules in liquid crystal is a powerful technique for determination of molecular structure and conformation. It is now known that a study of the nmr spectra of probe molecule dissolved in mixed liquid crystals of opposite diamagnetic anisotropies at a critical concentration and temperature provides two spectra corresponding to two orientations of the liquid crystal optic axis. In one of the orientation, the optic axis is preferentially aligned along the direction of the magnetic field and in the other it is orthogonal. The coexistence of the two spectra for the probe molecule has been theoretically interpreted and is based on the assumption that the probe molecule follows the orientation of the liquid crystal molecules. From the deutreron nmr spectra of specifically deuterated liquid crystals we now show that the coexistence of two spectra corresponding to both orientations also occurs in pure mixed liiquid crystals without any probe molecule. This result indicates that the probe molecules indeed follow the orientation of liquid crystal molecules. (H. J. C. Yeh, C. L. Khetrapal, A. Saupe, D. R. Boyd, D. M. Jerina, A. Brossi, X. D. Lo,).

THE DEVELOPMENT OF METHODS AND MATERIALS FOR THE STUDY OF MEDICAL PROBLEMS:

The objective of this project is to make contributions to the investigation of basic biological and medical problems by the application of chemical, physical and biological methods.

Cancer deaths frequently result from metastases. Of tumor cells which enter the circulation less than one percent are successful in negotiating the steps of metastasis. This vulnerability suggests that it should be possible to intercede at one or more steps of the process to reduce metastasis evern further. With that goal in mind the purpose of this work is to increase our understanding of the biology and chemistry of metastasis. The knowledge of cell biology generated will contribute to the investigation of other biological and medical problems.

Type IV collagenase (IV-Case) is specific for type IV collagen, the principal collagen of basement membrane (BM). Levels of the enzyme are elevated in metastatic tumor cells, and it may play a key role in the penetration of BM's during metastasis. Pretreatment of T241-PMT murine sarcoma cells with an antiserum prepared with murine IV-Case resulted in a dramatic decrease in lung metastases *iv* injection of the cells. The mechanism of this effect is being studied. An antiserum to the IV-Case from MCF-7 human mammary tumor cells has also been prepared.

The first barrier to extravasation encountered by circulating tumor cells is the endothelium; blood cells, soluble blood components, and the subendothelium are also involved in the process. A system using human amnion, with or without addition of endothelial cells, has been developed to facilitate study of the process *in vitro*. Further refinements of the system are being made. This system and measurements of proteolytic enzyme production, adhesion to extracellular matrices produced *in vitro* and to other surfaces, and of metastasis in mice are being used to study the effects of conditions of cell culture and chemical and biological treatment of cells on the metastatic potential. Understanding the mechanisms of such effects will increase our understanding of tumor cells in general and metastasis in particular. (C. M. Foltz, L. A. Liotta).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19001-12 LC

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Reactions and Immunochemistry of Carbohydrates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Cornelius P. J. Glaudemans, Chief, Section on Carbohydrates, LC, NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Carbohydrates

INSTITUTE AND LOCATION

NIH, NIADDK, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	4.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Section works on (1) Understanding the interaction between antibodies and antigens; (2) The preparation of antibodies with specificity for the determinants occurring on glycoproteins, and the characterization of the interaction of these determinants with their antibodies; (3) The section retains an interest in the structural of fungal capsules. Concerning (1) the Section is working on two aspects of elucidating the details of antigen/antibody interaction. Firstly, we have developed a system to study the influence of hydrogen bonding in ligand-antibody binding. To this effect we have prepared a large number of galactose oligosaccharides in which certain hydroxyl groups have been selectively replaced by fluoro groups. If hydrogen bonding comes about by ligand-hydrogen donation these saccharides would bind less if the particular (replaced) position had been involved. If protein-hydrogen donation were the driving force, affinity would increase or remain similar. In this way we have been able to delineate the nature of the binding bewteen a monoclonal antibody and its polymeric antigen in detail hitherto impossible to achieve. Secondly, we are trying to affinity-label the antibodies involved and this involves the synthesis of some complex reactive ligands. We are preparing oligosaccharidic (β1,6-linked galactosyl) derivatives with isothiocyanate or epoxypropyl as the reactive labelling group in order to label the monoclonal anti-galactan antibodies. Concerning (2) we have prepared eight synthetic immunogens bearing immunodeterminants known to occur on glycoproteins, and have obtained antibodies against several of these. At the moment we are purifying the antibodies and are trying to characterize them by affinity studies. These antibodies will be useful in characterization of glycoproteins on cell surfaces. Concerning (3) work was done on the immunodeterminants groups of aspergillus fumigatus, a pathogen in man.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 19200-34LC

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biological Testing for Antinociceptive Activity, Acute Toxicity and Dependence

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	A. E. Jacobson	Research Chemist	NIADDK-LC
	E. L. Atwell	Technician	NIADDK-LC
	M. Mattson	Technician	NIADDK-LC

COOPERATING UNITS *(if any)*
Departments of Pharmacology of the University of Michigan Medical School and the Medical College of Virginia, and the Committee on Problems of Drug Dependence,Inc.

LAB/BRANCH
Laboratory of Chemistry

SECTION
Medicinal Chemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.8	0.3	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Animal tests are used to determine the antinociceptive activity of potential analgesic agents. Acute toxicities have, also, been determined on a number of compounds with disparate biological activities. About 65 compounds have been sent to cooperating units for further investigation, under the auspices of the Committee on Problems of Drug Dependence.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19202-11LC

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Synthesis and Evaluation of Potential CNS, Antiinflammatory and Anticancer Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	K. C. Rice	Research Chemist	NIADDK-LC
	A. Brossi	Visiting Scientist	NIADDK-LC
	T. R. Burke, Jr.	Senior Staff Fellow	NIADDK-LC

COOPERATING UNITS *(if any)*

University of Colorado (B. Hoffer); NIADDK-LBC (P. Skolnick); NIMH-CP (S. Paul), University of Michigan (J. Woods). LBC-NCI (C. Klee), Oregon State University (J. White), LCS-NIMH (D. Lozovsky and I. Kopin)

LAB/BRANCH

Laboatory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Study of the effects of (+) and (-)-PCMP, an enantiomeric pair of phencyclidine derivatives, on blood pressure, heart rate and plasma prolactin in the rat revealed stereospecificity of action for (+)-enantiomer which was essentially equipotent with phencyclidine (PCP). Calmodulin was shown by covalent complex formation with newly-preapred [^{3}H] norchloropromazine isothiocyanate to have two phenothiazine binding sites and exhibited cooperative binding with phenothiazine. The calmodulin complex competitatively inhibit stimulation of phosphodiesterase by calmodulin but did not stimulate the enzyme.. Natural (-)-codine was synthesize from (+)-N-norreticuline using the biomimetic approach. The enantiomers of N-norreticuline were utilized for synthesis of several chiral 6'-methyl derivatives to examine stereospecificity of adrenergic and dopaminergic actions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19216-08LC

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure-Activity-Relationships of Colchinoids Based on Tubulin Binding

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Arnold Brossi	Visiting Scientist	NIADDK-LC
	Peter Kerekes	Visiting Scientist	NIADDK-LC

COOPERATING UNITS *(if any)*

C. F. Chignell, Research Triangle Park, North Carolina, Narayanan, NCI, Silver Spring, Maryland, K. Nakamura, Nippon Roche Research Center, Kamakura, Japan, J. V. Silverton, NIH, J. Flippen- Anderson, Naval Research Institute, Washington, D. C. T. MacDonald, University of Virginia, Charolottesville, Virginia, J. Wolff,

LAB/BRANCH NIADDK, NIH, Bethesda

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Modification of thiocolchicine following patterns established with colchicine, afforded analogs of thiocolchicine found highly potent in tubulin binding assays. In parallel reduced toxicity in mice and high potency in the P388 antitumor assay was detected with several analogs. Carbamates of deacetylthiocolchicines represent a novel group of potential antitumor and antigout compounds. Phenolic thiocolchicines and catecholic thiocolchicines were less potent in the tubulin binding assay. 1-Acetoxy- and 1,2-diacetoxy-analogs of thiocolchicine exist in solution as two isomers, possibly originating by atropisomerism. X-Ray diffraction of biologically potent and less potent colchicines was accomplished, showing little difference of the molecules in the solid state. Synthesis of thiodemecolcine and 3-demethyl-N-carbethoxydeacetylthiocolchicine was accomplished. Affinity labeling of colchicines by introducting spin-labels did not afford compounds useful for marking the colchicine binding site on tubulin. Esters of colchinoids with DADF were prepared, converted on tlc-plates after exposure to ammonia and iodine vapors into red colored and highyl visible erythrosyl derivatives.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 19219-07LC

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Synthesis of (±)-Hydroxy-6-keto-N-methylmorphinan and its O-methyl Ether

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	A. Brossi	Visiting Scientist	NIADDK-LC
	C. Chi-Shen	Visiting Fellow	NIADDK-LC

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Chemistry

SECTION
Medicinal Chemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.6	0.3	0.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Scale-up of our synthesis of (+)-2,4-dihydroxy-N-formyl-morphinan-6-one has been completed, affording enough material to prepare sufficient amounts of (+)- and (-)-4-methoxymorphinan for further testing.

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19223-07LC

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Tetrahydrpapaveroline ("THP")

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Arnold Brossi	Visiting Scientists	NIADDK-LC
	K. C. Rice	Research Chemist	NIADDK-LC
	F. L. Hsu	Senior Staff Fellow	NIADDK-LC
	H. Schmidhammer	Visiting Scientist	NIADDK-LC
	P. Sharma	Visiting Fellow	NIADDK-LC

COOPERATING UNITS *(if any)*
P. Skolnick and M. Schweri; J. V. Silverson and R. Potenzone, Jr., C. W. Abell, the University of Texas Medical Branch, Galveston.

LAB/BRANCH
Laboratory of Chemistry

SECTION
Medicinal Chemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.1	0.1	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Activity of optically active 1-benzyltetrahydroisoquinoilnes in dopaminergic and adrenergic assays is restricted to the S-isomer. Both racemic isoquinolines, 1-(o-hydroxybenzyl)-6-hydroxytetrahydroisoquinoline and racemic 1-(m-hydroxybenzyl)-6-hydroxytetrahydroisoquinoline showed good inhibitory activity in the α-adrenergic receptor assay and in the β-adrenergic receptor assay respectively.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19228-06LC

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Aromatic Oxidation and Antinociception in 6-Ketomorphinans

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)*

P.I.	Arnold Brossi	Visiting Scientist	NIADDK-LC
	H. Schmidhammer	Visiting Scientist	NIADDK-LC
	A. E. Jacobson	Research Chemist	NIADDK-LC
	L. Atwell	Technician	NIADDK-LC

COOPERATING UNITS *(if any)*

Judith L. Flippen-Anderson, Laboratory of the Structure of Matter, Navy Research Laboratory; H. Schmidhammer, Department of Chemistry, University of Innsbruck, Austria.

LAB/BRANCH

SECTION

Laboratory of Chemistry

INSTITUTE AND LOCATION

Medicinal Chemistry

TOTAL MAN-YEARS PROFESSIONAL OTHER

NIADDK, NIH, Bethesda, Maryland 20205

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects 0.1 ☐ (b) 0.1 Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Grewe-cyclization of o-hydroxybenzyl-substituted octahydroisoquinolines with refluxing hydrobromic acid afforded N-methylaporphinans as the final reaction products.

This project is discontinued.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19233-05LC

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Total Synthesis of Opioids via Dihydrothebainone and Derivatives

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)*

P.I.	K. C. Rice	Research Chemist	NIADDK-L:C

COOPERATING UNITS *(if any)*

Research Triangle Institute, Research Triangle Park, N. C. (F. I. Carroll)

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The NIH opiate total synthesis (previously reported) now renders the total synthesis of either enantiomer of codeine, morphine and thebaine possible in about 25% overall yield from m-methoxyphenethylamine with only 6-8 isolated intermediates. These results now provide, for the first time, a commercial source of medical opiates which is independent of the opium poppy. The route has now been extended to provide a practical, thebaine-free total synthesis of N-cycloalkylmethyl northebaine derivatives which are valuable intermediates for total synthesis of narcotic antagonist and agonist-antagonist drugs. Using commercially available m-methoxyphenethylamine as starting material either optical isomer of N-cyclopropylmethyl- and N-cyclobutylmethylnorthebaine can be synthesized in about 20% overall yield. Synthesis of a number of unnatural (+)-enantiomers of important thebaine derivatives required for pharmacological studies is in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19235-03LC

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Opiate Receptors Using Nonrigid Irreversible Inhibitors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	K. C. Rice	Research Chemist	NIADDK-LC
	A. E. Jacobson	Research Chemist	NIADDK-LC
	T. R. Burke, Jr.	Senior Staff Fellow	NIADDK-LC
	R. Lessor	Staff Fellow	NIADDK-LC

COOPERATING UNITS *(if any)*

W. Klee, NIMH, NIH, Bethesda, Md., R. Rothman, C. Pert, NIMH

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.4	0.4	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In a continuation of our study into the structure and function of the opiate receptor system, the first enantiomeric pair of alkylating opiates have been prepared. These enantiomers have been shown to exhibit substantial stereoselectivity for the delta opiate from NG108-15 cells. A mu selective ligand based on etonitazene has been tritiateded to high specific activity. Other alkylating opioids have been prepared and characterized biochemically.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19236-03LC

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Opioid Receptors Using Rigid Probes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I.	A. E. Jacobson	Research Chemist	NIADDK-LC
	R. Lessor	Staff Fellow	NIADDK-LC
	K. C. Rice	Research Chemist	NIADDK-LC
	W. A. Klee	Research Biochemist	LGCB-NIMH-ADAMHA

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A series of relatively rigid molecules, from the endoethenooripavine family of opioids known to bind to opioid receptors from rat brain cerebrum homogenates and neuroblastoma-glioma hydride cells, were converted to ligands potentially capable of irreversible interaction with those receptors. Two other compounds, besides our initial ligand in that series, have now been found to act as an irreversible binding ligand for the delta and mu opioid receptors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 19237-03LC

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Topological Characterization of Opiate Receptors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	K. C. Rice	Research Chemist	NIADDK-LC
	T. R. Burke, Jr.	Staff Fellow	NIADDK-LC
	A. E. Jacobson	Research Fellow	NIADDK-LC

W. Klee, NIMH, Bethesda, MD

COOPERATING UNITS *(if any)*

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Laboratory of Chemistry

SECTION
Medicinal Chemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.2	0.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Conformationally restricted congeners of the opiate agonist 5-m-hydroxyphenyl-2-methylmorphan were prepared as probes to examined topological requirements for in vitro binding to opiate receptor subpopulations. Two congeners were inactive in vivo as agonists or antagonists and did not bind appreciably to opiate receptor preparations. One congener was a weak antagonist with no agonist properties and had a weak binding constant for opiate receptors. Work is presently progressing on the synthesis and biological evaluation of further members of this series.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 19239-03LC

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Isolation and Characterization of the Antitrypanosomal Factor from P. fluoresens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)*

P.I.	K. C. Rice	Research Chemist	NIADDK-LC
	T. R. Burke, Jr.	Senior Staff Fellow	NIADDK-LC

COOPERATING UNITS *(if any)*
LPD, NIAID (T. Mercado)

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SECTION
Medicinal Chemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER:
0.2	0.2	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Pseudomonas fluorescens was recently observed to secrete a factor which is toxic for Trypanosoma cruzi, the causative organism for South American sleeping sickness (Chagas' Disease). We have isolated and identified several components which are active against T. cruzi but are also hemolytic. Other biological studies are planned.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19241-03LC

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Agonists and Antagonists for the Phencyclidine Receptor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	A. E. Jacobson	Research Chemist	NIADDK-LC
	M. Mattson	Technician	NIADDK-LC
	P. Huguenin	Visiting Fellow	NIADDK-LC
	M. Rafferty	Guest Worker	NIADDK-LC
	K. C. Rice	Research Chemist	NIADDK-LC

COOPERATING UNITS *(if any)*

Experimental, Therapeutics Branch, NINCDS, NIH (Drs. T. O'Donohue, P. Contreras)
Department of Pharmacology, University of Michigan Medical School (Drs. J. Woods).

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.7	1.2	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An irreversible ligand (Metaphit), specific for phencyclidine receptors in vitro, has been found which can block phencyclidine's pharmacological effects 24 hours after its icv introduction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19243-03LC

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Novel Approaches Towards the Synthesis of Pyrrolizidine-Ant-Alkaloids

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	A. Brossi	Visiting Scientist	NIADDK-LC
	K. Takahashi	Visiting Scientist	NIADDK-LC
	W. Gessner	Visiting Fellow	NIADDK-LC

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Stereoselective synthesis of (Z)-5-alkyl-pyrrolizidine-3-ones was achieved from Sorm's dilactam.

This project is temporarily discontinued.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19244-02LC

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

(+)-Morphinans and (+)-Opioids as Antitussive Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	A. Brossi	Visiting Scientist	NIADDK-LC
	K. C. Rice	Research Chemist	NIADDK-LC
	A. E. Jacobson	Research Chemist	NIADDK-LC
	H. Schmidhammer	Visiting Scientist	NIADDK-LC
	F. L. Hsu	Senior Staff Fellow	NIADDK-LC
	C. Chi-Shen	Visiting Fellow	NIADDK-LC

COOPERATING UNITS *(if any)*

Y. Yagi, Roche Research Center, Kamakura, Japan

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Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 0.5

PROFESSIONAL: 0.5

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Rac. 6-ketomorphinans and (+)-6-keto-N-methylmorphinan of the unnatural series showed pronounced antitussive activity. A synthesis of 4-oxygenated ketomorphinans of unnatural configuration has been initiated. Optical resolution of the required tetrahydroisoquinoline intermediate has been achieved. Racemizaton of the unwanted optically active isomer has also been accomplished.

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19245-02LC

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chemically Induced Parkinson's Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	A. Brossi	Visiting Scientists	NIADDK-LC
	W. Gessner	Visiting Scientist	NIADDK-LC

COOPERATING UNITS *(if any)*

C. W. Abell, Human Biological Chemistry and Genetics, The University of Texas Medical Branch, Galveston, Texas

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Substantial quantities of 4-phenyl-N-methyl-1,2,3,6-tetrahydropyridine claimed to produce Parkinson's disease in monkeys have been prepared. The 4-chlorophenyl analog has also been prepared in form of the secondary and N-methylated amine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Zo1 AM 19246-02LC

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Opiate Receptors Using Positron Emission Transaxial Tomography

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	K. C. Rice	Research Chemist	NIADDK-LC
	T. R. Burke, Jr.	Senior Staff Fellow	NIADDK-LC

COOPERATING UNITS *(if any)*

NIMH (C. Pert), CC (S. Larson, W. Eckelman)

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A synthetic sequence for introduction of position-emitting ^{18}F into the carbon-nitrogen skeleton of 17-methyl or 17-cyclopropylmethyl-3,14,-dihydroxy-4,5-epoxymorphinan was developed. The potent opiate agonist oxymorphone and antagonist naltrexone respectively, were the prototypes for N-methyl and N-cyclopropylmethyl fluoro derivatives. The agonist was tritiated to high specific activity and preliminary experiments indicate successful synthesis of the ^{18}F labeled morphinans.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19247-02 LC

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Perhydrohistrionicotoxins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	A. Brossi	Visiting Scientist	NIADDK-LC
	K. Takahashi	Visiting Scientist	NIADDK-LC
	W. Gessner	Visiting Fellow	NIADDK-LC

COOPERATING UNITS (if any)

E. X. Albuquerque, Department of Pharmacology and Experimental Therapeutics, Universtiy of Maryland, Baltimore.

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.0	0.8	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Total synthesis of natural (-)-perhydrohistrionicotoxin, its unnatural (+)- antipode, (-)-2-depentylperhydrohistrionicotoxin and its (+)-antipode have been achieved. The optical isomers do not show great quantitative differences in frog muscle preparations. A synthesis of (±)-unalkylated spiroaminoalcohols and (±)-7-debutylperhydrohistrionicotoxin has also been achieved.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19248-01LC

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antitussive Morphinans: Structure-Activity Relationships

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)

P.I.	Arnold Brossi	Visiting Scientists	NIADDK-LC
	Peter Kerekes	Visiting Scientists	NIADDK-LC

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.7	0.7	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Synthesis of (+)-4-methoxy-substituted Ketomorphians to be evaluated as antitussive agents and to afford information regarding the existence of a possible antitussive receptor has been continued. A variation of a synthetic scheme developed ealier has afforded (+)-4-methoxy-morphinan-6-one, a crucial intermediate for obtaining the desired ketomorphinans of unnatural configuration. Asymmertic reduction of a 3,4-dihydroisoquinoline intermediate with a complex of sodiumborohydride with a proline derivative derived from natural S-proline has afforded the desired optically active tetrahydroisoquinoline of S-configuration. The original synthesis developed with racemic material will now be extended to the optically active S-enantiomer.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19249-01LC

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies Regarding the Neurotoxin 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine(MPTP)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	A. Brossi	Visiting Scientist	NIADDK-LC
	Wieslaw Gessner	Research Fellow	NIADDK-LC

COOPERATING UNITS *(if any)*

C. W. Abell, Dept. Human Biol. Chem. & Genetics, Division of Biochem., University of Texas Medical Branch at Galveston; S. P. Markey, Institute of Mental Health, Lab. of Chemistry.

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIH, NIADDK, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.1		0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
  ☐ (a1) Minors
  ☐ (a2) Interviews

☐ (b) Human tissues

☒ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Analogs of MPTP, substituted in the phenyl ring with chlorine or hydroxy groups were synthesized. The synthesis also afforded piperidine and pyridine congeners of MPTP. The compounds were evaluated in vitro as inhibitors of dihydroteridine reductase. The most active inhibitor was 4-(3',4'-dihdroxyphenyl)-pyridine. The N-oxide of MPTP may constitute an intermediate in the conversion of MPTP to 4-phenylpyridinium salts, detected as major metabolites of MPTP in the brain of monkeys. The N-oxide of MPTP converted with anhydrides in vitro into dihydro intermediates which smoothly aromatized to pyridinium salts with air and Pt catalyst. The dihydro intermediates could also be aromatized with the N-oxide of MPTP. The N-oxide of MPTP also oxidizes in vitro dopamine into 6-hydroxydopamine. It will now be explored whether the N-oxide of MPTP is being formed in animals or man.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19250-01LC

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chemistry and Metabolism of Qinghaosu, a Chinese Antimalarial Drug

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Arnold Brossi	Visiting Scientist	NIADDK-LC
	X. Luo	Guest Scientist	NIADDK-LC

COOPERATING UNITS *(if any)*

C. Canfield, Walter Reed Army Research Institute, Silver Spring, Maryland.

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Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIH, NIADDK, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Artemether and artesunic acid, two synthetic Chinese antimalarials derived from the Chinese drug qinghaosu from Artemisia were characterized and their configuration established. Dihydroqinghaosu and its epoxy analog, both metabolites of qinghaous in man, were converted into DADF-ester derivatives, analyzed on tlc-plates after exposure to ammonia and iodine as red colored erythrosyl derivatives. This is a highly sensitive assay allowing detection of 0.1 - 1 λ of material.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19251-01LC

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure-Activity-Relationships of Perhydrohistrionicotoxin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)*

P.I.	Arnold Brossi	Visiting Scientist	NIADDK-LC
	W. Gessner	Research Fellow	NIADDK-LC

COOPERATING UNITS *(if any)*

E. X. Albuquerque, Medical School, University of Maryland at Baltimore.

LAB/BRANCH

Laboratory of Chemistry

SECTION

Medicinal Chemistry

INSTITUTE AND LOCATION

NIH, NIADDK, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.5	0.5	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Perhydrohistrionicotoxins not alkylated in the spiro ring system are now available by straight forward chemistry. They are being explored as agonists of the acetylcholine receptor. Alkylation of the basic lactam moiety, obtained from "naked" perhydrohistrionicotoxin by oxidation, afforded, depending on the reaction conditions two alkylated products. Their chemistry is being explored.

This project is terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19401-19 LC

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Natural Products as AGonists, Antagonists, Desensitizers & Probes for Receptors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)*

Dr. Bernhard Witkop, Chief, Laboratory of Chemistry, NIADDK

COOPERATING UNITS *(if any)* O.Yonemitsu, Y.Kanaoka & T.Iwakuma, Univ. Hokkaido, Japan; M. Nakagawa, Chiba Univ., Japan; I.L.Karle, U.S. Naval Res. Lab., Wash.D.C.; E.X. Albuquerque, C.Spivak & M.P. Blaustein, Univ. Md. Med. Sch; E. Gössinger, Univ. Vienna, Austria; R. Aronstam, Univ. Ga.; E.M. Kosower, Tel-Aviv Univ., T. Gund, N.J. Inst. Tech., Newark, N.J.

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Metabolites

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
.5	.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Chief Investigator - largely with extramural support - has kept up a widely diversified program, international and interdisciplinary in character, involving binding studies of agonists, electrophysiology of ion flux, photochemistry of psychoactive drugs, modeling of nicotinic and muscarinic agonists, consultation on protective measures against organophosphorous agents--and support function for a clinical program on degenerative diseases of the brain. In addition the Chief Investigator is active in international scientific exchange and collaboration with most countries of Western Europe, China, Japan and Taiwan.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19402-11 LC

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Interferon Induction and Action. The Antiviral Activity of Nucleoside Analogs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Paul F. Torrence, Research Chemist, LC, NIADDK

COOPERATING UNITS *(if any)* D.Eppstein,Syntex Res.;J.Content,Inst.Pasteur,Gelgium;E.DeClercq, Rega Inst.,Belgium,W.P.Pfleiderer,U.Konstanz,W.Ger.;H.Sawai,U.Tokyo,Japan;J.-L. Imbach,U.Montpellier,France;W.Dawson,U.Cal.;M.I.Johnston,USUHS;R.Silverman,USUHS; H.Friedman,U.S.Fla.,Tampa;K.Schram,U.Ariz.,Tucson

LAB/BRANCH
Laboratory of Chemistry

SECTION
Section on Metabolites

INSTITUTE AND LOCATION
NIH,NIADDK, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
4.3	4.0	.3

CHECK APPROPRIATE BOX(ES)

XX (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Interferon-induced enzyme activities such as the oligo(2'→5')adenylate synthetase, the 67K dalton protein kinase and oligo(2'→5') A phosphodiesterase are investigated with a goal of understanding their role in the action of interferon, the induction of interferon by double-stranded RNA and, perhaps, control of cell growth and differentiation. Analogs of the mediator of interferon action, 2-5A, are synthesized in order to define the relationship between oligonucleotide structure and binding to and activation of the 2-5A dependent endonuclease with the eventual goal of designing useful chemotherapeutic agents based on this system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19408-11 LC

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Semirigid Agonists at the Nicotinic Acetylcholine Receptor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

James A. Waters, Research Chemist, LC, NIADDK

COOPERATING UNITS *(if any)*

Addiction Research Center, NIDA (Dr. C. E. Spivak); Univ. of Md. School of Med., Baltimore, MD (Dr. E. X. Albuquerque); Newark College of Eng. & Chem., Newark, New Jersey (Dr. T. Gund)

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Metabolites

INSTITUTE AND LOCATION

NIH, NIADDK, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

3-Acetyl-3,4-dehydropiperidines and related cyclic analogs of anatoxin-a have been synthesized for study of the nicotinic acetylcholine receptor. Arecolone and (-)-ferruginine methiodides are active nicotinic agonists in the rectus abdominus muscle assay. (-)-Nor-ferruginine, a homolog of the potent agonist anatoxin displayed weak activity. 4-Diphenylacetoxy-1-methylpiperidine (4-DAMP)-type compounds have been synthesized for selective action at the muscarinic acetylcholine receptor sites (M_1 & M_2).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19411-02 LC

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

A Biomimetic Approach to the Synthesis of Ergotamine Peptides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Thomas F. Spande, Research Chemist, NIADDK-LC

COOPERATING UNITS *(if any)*

Dr. H. C. J. Ottenheijm, Laboratory of Organic Chemistry,
Catholic University, Nijmegen, The Netherlands

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Metabolites

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0		

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been transferred to Z01 AM 3110-19 LBC

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19603-08 LC

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Fluoro Analogs of Enzyme Substrates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Louis A. Cohen, Chief, Section on Biochemical Mechanisms, LC, NIADDK

COOPERATING UNITS *(if any)*

P. Dannies, Dept. of Pharmacology, Yale Medical School, New Haven, Conn.
G. Feuerstein, Dept. of Pharmacology, USUHS
E. DeClercq, Louvain, Belgium

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Biochemical Mechanisms

INSTITUTE AND LOCATION

NIADDK-LC

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.1	1.4	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Pursuant to our discovery (1971) of a photochemical route to fluoroimidazoles, we have found that various fluoro analogs of bioimidazoles (histamine, histidine, etc) show a variety of useful properties as substrates and inhibitors. For example, 2-fluoro-L-histidine not only is incorporated into microbial and animal protein in place of histidine, but also serves as an antibacterial, antiviral and antileukemic agent. Recent studies with labelled amino acids reveal that this analog also limits the uptake of several other amino acids into the cell. These, and other, biological properties appear to be specific for the 2-fluoro isomer. 5-Fluoro-Im-TRH failed to bind to rat pituitary cells in vitro and failed to stimulate prolactin release. On the other hand, microinjection of this compound into the rat hypothalamus results in strong elevations in heart rate and blood pressure. Thus, the long-sought separation of the pituitary and CNS activities of TRH have been achieved by means of a fluoro analog. Additional analogs are being synthesized in the hope of achieving stability to hydrolytic breakdown without loss of selectivity. Fluoroimidazoles are obtained by photochemical replacement of amino groups and aminoimidazoles are generally prepared by reduction of arylazoimidazoles. Thus, 4-fluoro-2-aminoimidazole-5-carboxamide was prepared and was converted into the 2,4-difluoro derivative. Surprisingly, the isomeric 2-fluoro-4-aminoimidazoles are far less stable and must be stored at very low temperatures. The N-trifluoroacetyl derivative, however, is quite stable in the solid state at 25°C. When the latter compound is dissolved at pH 7.4 and 37°C, the trifluoroacetyl group is lost spontaneously ($t_{1/2}$ = 3 hr) and the potential antiviral agent is released in situ. In parallel with the broad-spectrum antiviral activities already shown by 4-fluoroimidazole-5-carboxamide, the new compounds will also be investigated for such activities.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19604-14 LC

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
General Principles of Enzyme Catalysis and Simulation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Louis A. Cohen, Chief, Section on Biochemical Mechanisms, LC, NIADDK

COOPERATING UNITS *(if any)*
Eugene Man, University of Miami
Oshio Ueno, Nagoya, Japan

LAB/BRANCH
Laboratory of Chemistry

SECTION
Section on Biochemical Mechanisms

INSTITUTE AND LOCATION
NIH, NIADDK, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.5	0.3	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In order to account for the remarkable catalytic power of enzymes, it is generally considered that the activation free energy (the energy hill which must be surmounted to get from starting material to product) is contributed both by binding of the substrate to the enzyme (step 1) and by chemical manipulation of the bound substrate (bond-making and breaking, step 2). Popular opinion holds that most of the activation energy is supplied in step 2: We have proposed, however, that the overall catalytic process can be explained more reasonably if it is assumed that the first step (binding) contributes a more significant, and sometimes major, share of the activation energy. To support this theory, we have synthesized a large variety of test-tube models which simulate the bound substrate by being frozen into a single, favorable conformation and by having the interacting groups brought into the closest possible juxtaposition (stereoopulation control). These compounds undergo intramolecular reactions at rates comparable to those catalyzed by enzymes, sometimes even too fast to measure. The protein raises both the entropic and enthalpic components of the substrate by binding it in a single, rigid conformation. To prove that our model compounds are truly frozen in unique conformations in solution, we have used temperature-variable NMR studies, x-ray crystallography, computer calculations of free energy content and kinetic analysis. Thus, we have found that log k for enhanced cyclization is a function of the Van der Waals size of restricting groups and is independent of electronic effects. More recently, we have initiated efforts to demonstrate that these molecules possess molecular chirality, and that the resolved enantiomers do not racemize at elevated temperatures. Indeed, some of our models show such slow rotation of single bonds (half-life = 20 minutes), that conformational change becomes the rate-limiting step in cyclization.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 19605-08 LC

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chemistry of Substituted Imidazoles

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Louis A. Cohen, Chief, Section on Biochemical Mechanisms, LC, NIADDK

COOPERATING UNITS *(if any)* H. Kimoto, Industrial Res. Inst., Nagoya, Japan; R. Henkin, Georgetown Univ. Hosp., Wash. D.C.; E. De Clcrcq, Louvain Univ., Belgium; A. Shanzer, Rehovot, Israel; W. Nagai, Nagoya, Japan; G. Feverstein, USUHS; D. Lozovsky, LCS, NIMH

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SECTION
Section on Biochemical Mechanisms

INSTITUTE AND LOCATION
NIADDK-LC

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.2	1.5	0.7

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Pursuant to our discovery (1971) of a photochemical route to fluoroimidazoles, we have found that various fluoro analogs of bioimidazoles (histamine, histidine, etc.) show a variety of useful properties as substrates and inhibitors. For example, 2-fluoro-L-histidine not only is incorporated into microbial and animal protein in place of histidine, but also serves as an antibacterial, antiviral and antileukemic agent. Recent studies with labelled amino acids reveal that this analog also limits the uptake of several other amino acids into the cell. Such results provided an impetus to develop synthetic methods for other new analogs of histamine and histidine with substituents at C-2, C-4 or both. Analogs containing all the major functional groups are now on hand. Synthesis of 2-halohistidines proved especially difficult: a general procedure has now been developed in which 2-diazohistidine is decomposed in the presence of halide ion and copper powder. Quite surprisingly, 2-iodohistidine is active against microorganisms which are unaffected by 2-fluorohistidine. The azido analogs are of interest for photoaffinity labelling of receptors and enzymes.

The trifluoromethyl analogs are labile in mildly alkaline media, making them potentially useful as receptor affinity labels for use in vivo. Toward this end, the isomeric trifluoromethylhistidine analogs of TRH have been synthesized and are undergoing biological evaluations. Although 2-CF_3-His-TRH fails to bind to pituitary cells in vitro and fails to release prolactin in this system, the compound does release prolactin in vivo; this result suggests that the brain contains nonpituitary receptors for TRH which also stimulate prolactin release. The use of TRH for diagnosis of hypothalamic dysfunction is limited by its powerful cardiovascular effects--large increases in heart rate and blood pressure. The 2-CF_3 analog is 5-6 times as effective as TRH in promoting prolactin release but has no significant cardiovascular effects--thus making it a potentially valuable diagnostic reagent.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 19606-08 LC

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Halogenated Biogenic Amines in Biochemistry and Pharmacology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Kenneth L. Kirk, Research Chemist, NIADDK, LC

COOPERATING UNITS *(if any)* C. R. Creveling (LBC,NIADDK); C. C. Chieuh, R. Borwn, J. Costa (NIMH); I. Kopin (NINCDS); M. Linnoila, T. Brown (NIAAA); D. Thakker (NCDB); M. Channing, W. Eckelman (Clinical Ctr., Dept. of Nuclear Medicine); A. Shanzer, K. Muszkat (Weizman Institute)

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Laboratory of Chemistry

SECTION
Section on Biochemical Mechanisms

INSTITUTE AND LOCATION
NIH, NIADDK, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER:
1.8	0.6	1.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Biogenic amines play key roles in neurotransmission, metabolism, and in control of various physiological processes. Progress has been slow in determining their mechanisms of transport, storage, release and mode of action. Ring-halogenated analogs have proved to be powerful tools for the study of these mechanisms, since they simulate the geometries of the natural compounds so well. By virtue of its very small size and high electronegativity, fluorine should be a very favorable replacement for hydrogen in these analogs. Some years ago, we developed novel methods for the synthesis of such analogs and have prepared a wide variety of biogenic amines with fluorine at various ring positions. The biological properties and usefulness of these analogs have proved unexpectedly rewarding and continue to find application in a multitude of studies. Perhaps the most significant finding, to date, is that 2-fluoronorepinephrine is a pure β-adrenergic agonist, while the 6-fluoro isomer is a pure α-adrenergic agonist. Various explanations for the role of fluorine in creating such specificity have been considered and discarded. We now propose that a critical ion-dipole interaction exists between a positively-charged site on the receptor and an electron-rich carbon atom of the benzene ring. Inductive withdrawal by fluorine would weaken this interaction and produce the observed specificities. Results from pharmakokinetic studies of ring-fluorinated catecholamino acids demonstrate their potential as biological tracers, including use as ^{18}F-Pet scanning agents. A series of fluorinated analogs and homologs of biogenic amines and metabolites are ideal standards for new assays of biological samples using HPLC and electrochemical detection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19607-02 LC

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chemistry, Biochemistry and Pharmacology of Bioindole Analogs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)*

Louis A. Cohen, Chief, Section on Biochemical Mechanisms, LC, NIADDK

COOPERATING UNITS *(if any)*

Edith Miles, LBP, NIADDK

LAB/BRANCH

Laboratory of Chemistry

SECTION

Section on Biochemical Mechanisms

INSTITUTE AND LOCATION

NIADDK-LC

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.2	1.0	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Tryptophan is an essential amino acid, serving as the precursor of the neurotransmitter, serotonin, and of the hormone, melatonin, in addition to its roles in enzymes and in receptor proteins. Tryptophan is metabolized in mammals by a pyrroloxygenase in the liver, where it can serve as a precursor of nicotinamide (Vitamin B_6) in some animals. In other tissues, tryptophan and related indoles are metabolized by a distinct oxygenase, the activity of which is dramatically increased (up to 100-fold) upon administration of bacterial lipopolysaccharides or interferon. The role of this oxygenase in the response of the organism to infection is unknown, however. We anticipated that certain 2-substituted tryptophans might serve as selective "suicide substrates" for these oxygenases. Analogs of tryptophan with electronegative substituents at C-2 had not been previously prepared. We have obtained 2-chloro- and 2-bromo-L-tryptophan by radical halogenation, and 2-nitro-L-tryptophan as a minor product of direct nitration. The mechanisms of hydrolysis of the 2-halotryptophans at low pH have now been fully elucidated and reveal the involvement of intramolecular proton transfer in the conversion of the stable indole to the labile indolenine tautomer. An enzyme carboxyl group should also promote indolenine formation, suggesting the indolenine to be the true substrate for tryptophan enzymes. Indeed 2-oxindolyl-L-alanine and 2,3-dihydrotryptophan are excellent competitive inhibitors of these enzymes. This unique catalytic power of the carboxyl is now being utilized to catalyze the replacement of the 2-halo group by other nucleophiles in aprotic solvents. The availability of the novel 2-nitrotryptophan now provides an alternative route to other 2-substituted tryptophans--of potential value as affinity and photoaffinity labels, and as antibacterial agents. 5-Azido-L-tryptophan has already been found effective as a photoaffinity label for tryptophan synthase. The properties and hydrolysis mechanisms of 2-haloindole-3-acetic acids contrast markedly with those of the analogous propionic acids.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19608-01 LC

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Functionalized Congeners of Bioactive Compounds

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: K. Kirk, Research Chemist, LC, NIADDK

Other: K. Jacobson, Staff Fellow, LC, NIADDK

COOPERATING UNITS *(if any)* P. Marangos, D. Jakobowitz (NIMH), J. Daly (NIADDK), R. Blasberg (NCI), W. Eckelman (CC NM)

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Laboratory of Chemistry

SECTION

Section on Biochemical Mechanisms

INSTITUTE AND LOCATION

NIADDK-LC

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.2	1.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Neurotransmitters and other neuromodulators are derivatized as functionalized congeners for the purpose of receptor studies and designing new, therapeutic agents. The functionalized congeners are designed to be potentially reactive towards drug carriers, molecular probes for the receptor, or solid supports. Functionalized congeners of adenosine receptor agonists and antagonists (di-alkylxanthines) have been synthesized. The congeners bear an amino or a carboxylic acid group and can be linked to carriers to form conjugates of high affinity for the receptor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19801-39LC

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Service Functions and Instrumentation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. Dr. David F. Johnson, Chief, Microanaly. Ser. and Instru., NIADDK/LC

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Chemistry

SECTION

Microanalytical Services and Instrumentation

INSTITUTE AND LOCATION

NIH, NIADDK, Bethesda, Maryland 20205

TOTAL MAN-YEARS 5 PROFESSIONAL 2 OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Basic research and service functions are performed by members of the Section. A major mission of the organization involves the instrumental and chemical analyses provided to scientists of the Laboratory of Chemistry, Laboratory of Bioorganic Chemistry, NIH, and to a limited extent to personnel of other government agencies. Approximately 30 of the more common elements and five functional groups are determined on a quantitative basis, using ultramicro, micro, and semi-micro techniques as required. The materials analyzed include organic and inorganic research samples, commercial preparations and various biological specimens. Instrumental analyses include: GC/MS spectrometry, gas-liquid chromatography, GC with radioactive monitoring, infrared, nuclear magnetic resonance amino acid analysis, ultraviolet and flame photometry. Assitance in the interpretation of spectra is rendered on request.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19802-11LC

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of a Secondary Alcohol Dehydrogenase in Tetrahymena Furgasoni

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Nancy Lamontagne	Research Chemist	NIADDK-LC

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemistry

SECTION

Microanalytical Services and Instrumentation

INSTITUTE AND LOCATION

NIADDK, nih, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues xx (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19803-11LC

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Applications of NMR in Biochemical and Biological Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Dr. Herman Yeh	Research Chemist	NIADDK/LC
	D. M. Jerina	Sec.Chief	NIADDK-LBC
	A. Brossi	Sec.Chief	NIADDK-LC
	Xuan-de Luo	Guest Worker	NIADDK-LC
	P. Kovac	Visiting Assoc.	NIADDK-LC
	C.P.J. Glaudemans	Sec. Chief	NIADDK-LC

COOPERATING UNITS *(if any)*

C. L. Khetrapal (Raman Research Inst., India); A. Saupe (Liquid Crystal Instr., Kent State Univ.,Kent,OH); D.R. Boyd(Queens Univ. of Belfast, N. Ireland);

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Laboratory of Chemistry

SECTION

Microanalytical Services and Instrumentation

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
0.5	0.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to develop and apply nuclear magnetic resonance for elucidating molecular structure and for studying the interactions within and between molecules, especially those of biological interests.

An empirical method has been developed for determining the optical purity of a range of epoxides derived from both cyclic and acyclic olefins and arene oxides using nmr in the presence of chiral lanthanide shift reagents. This method is found to be useful for predicting the absolute stereochemistry of non-K-region and bay region tetrahydro epoxides and K-region arene oxides.

Deuteron nmr spectra of specifically deuterated liquid crystals have been investigated. The resutls show a switch-over transition taking place at a critical concentration and temperature in mixed liquid crystals of opposite diamagnetic anisotropies.

Various nmr methods are also employed to determine the structures of qinghaosu and its analogs (antimalarial drugs), and a nubmer of fluorinated carbohydrate derivatives related to (1z6)-ç-D-galactopyranan which is planned for the binding study of saccharides to immunoglobulins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 19804-11LC

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Development of Methods and Materials for the Study of Medical Problems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I. Calvin M. Foltz, Research Chemist, NIADDK/LC

COOPERATING UNITS *(if any)*
National Cancer Institute (Lance A. Liotta, Pathologist, NCI/LP)

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Laboratory of Chemistry

SECTION
Microanalytical Services and Instrumentation

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The primary goal of this work is to contribute to the investigation and solution of basic biological and medical problems by the application and development of chemical, physical and biological methods. This is being done by the application of chemistry to the isolation and study of biomolecules, by the synthesis and study of chemical compounds, and by the study of the interactions of chemical and biologicals with normal and tumor cells in vitro and with tumors and tumor cells in vivo in mice.

Areas of special interest are organic chemistry, biochemistry, medicinal chemistry, cell biology, tissue culture, cancer biology, cancer chemotherapy, and selenium chemistry.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 19806-09LC

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Nature of Steroid-Receptor Interactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. S. Stoney Simons, Jr., Research Chemist, NIADDK/LC

COOPERATING UNITS *(if any)*

E. I. B. Thompson, NCI; H. J. Eisen, NICHHD

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Laboratory of Chemistry

SECTION

Microanalytical Services and Instrumentation

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.2	3.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is to define the initial, intra-cellular events of steroid hormone action. Synthetic glucocorticoid derivatives, some of which react to form covalently labeled receptors via affinity labeling, have been prepared and are being used with glucocorticoid-responsive rat hepatoma tissue culture cells to examine: (1) steroid-receptor binding site interactions; (2) the effects of steroid binding on receptor conformation; and (3) the nature of "activation" of receptor-steroid complexes. We have combined affinity labeling by dexamethasone 21-mesylate with protease digestion in order to study various physical states (i.e., activated and unactivated) of covalent receptor-steroid complexes and to assess receptor-homology in different target tissues. These studies indicate that if activation of glucocorticoid receptor-steroid complexes is accompanied by changes in conformation or oligomerization (as has been suggested by others), the changes are not detectable by these methods and must be of limited magnitude. We have also synthesized a highly fluorescent glucocorticoid derivative (Dex-C_2-Rho). This steroid yielded the first specific, fluorescent glucocorticoid receptor-steroid complex, which should be very useful in the study of receptor-steroid complexes by fluorescence spectroscopy.

ANNUAL REPORT
LABORATORY OF CELL BIOLOGY AND GENETICS

The Laboratory of Cell Biology and Genetics pursues a broad spectrum of studies on cell functions ranging from cells and tissues to subcellular organelles, chromosomes, and genes.

The primary interest of the section on Cell Biology and Biochemistry is to discover the molecular basis for regulation of cellular secretion of hormones and transmitters by exocytosis. Our approach has been to search for factors mediating hormone synthesis and assembly into secretory vesicles, calcium action, membrane-cytoskeletal interactions, the formation of complexes between secretory vesicles and plasma membranes, and the final breakage (fission) of this complex leading to the ultimate release event of exocytosis.

Cultured adrenal chromaffin cells provide an excellent system for investigating regulation of catecholamine biosynthesis and release. To this end, we have developed a HPLC reverse-phase system to separate tyrosine, the catecholamines (dopa, dopamine, norepinephrine, epinephrine), and their major metabolites (DOPAC, HVA, normetanephrine, VMA, metanephrine, and DOPEG). Treatment of the cells with a battery of secretagogues, including nicotine, high potassium, veratridine, and oubain caused increases in the biosynthetic rates of the catecholamines that varied in magnitude among the compounds tested. In addition, the tested secretagogues varied in the specificity of their released products, suggesting differences in their mechanisms of release. The activity of the adrenal medullary enzyme, phenylethanolamine N-methyl transferase (PNMT) for synthesis of adrenaline, is dependent on glucocorticoids secreted from the adrenal cortex. The mechanism of how this cell responds to glucocorticoids, however, has only been recently discovered after we were able to reproduce hormone sensitivity in culture. The chromaffin cell, curiously, has a glucocorticoid receptor that is 10-fold higher in sensitivity than similar proteins in other tissues, and it seems to act on PNMT by a non-nuclear mechanism. Ascorbic acid is the optimal electron donor for isolated dopamine beta hydroxylase, but the importance of this observation to dopamine beta hydroxylase function in chromaffin granules has been open to question.

Chromaffin granules incubated with (^{3}H) dopamine and Mg-ATP were able to transport catecholamine and to synthesize (^{3}H) norepinephrine. With the addition of ascorbic acid, chromaffin granules doubled the amount of (^{3}H) norepinephrine biosynthesis as compared to control. However, ascorbic acid specifically enhances norepinephrine biosynthesis from dopamine in isolated chromaffin granules, without actual uptake of ascorbic acid, indicating the existence of an electron transport system across the chromaffin granule membrane. Ascorbic acid itself was found to be secreted from cultured bovine adrenal chromaffin cells coincidentally with catecholamines using a variety of secretagogues. However, while catecholamines were secreted from granules, ascorbate came from the cytosol. The problem of how integrated synthesis of catecholamines can occur when movements of enormous magnitude across granule membranes must occur has also been resolved by evidence that two of the enzymes involved in catecholamine biosynthesis, tyrosine hydroxylase (TH), and phenylethanolamine N-methyl transferase (PNMT), classically considered to be soluble proteins, may actually be localized on the outer surface of the chromaffin granule. DBH is, of course, within the granule in any event. In addition to utilization for catecholamine biosynthesis and capacity to be secreted, adrenal chromaffin cells have been found to accumulate ascorbic acid by a saturable high affinity mechanism that is inhibited by hydrocortisone. Since both ascorbic acid and hydrocortisone are secreted from cortical cells during stress into the adrenal portal system and thus contact medullary chromaffin cells, we suggest that the control of ascorbic acid uptake by hydrocortisone indicates the existence of a heretofore undescribed regulatory mechanism.

Secreting chromaffin cells were tested for faithfulness to the chemiosmotic hypothesis for exocytosis, proposed by us in previous work, to account for energetics of exocytosis. The cells corresponded to the predictions of the hypothesis in many ways, including sensitivity to external osmotic strength, variation in many external anions, and other drugs. However, stilbene disulfonates were inactive and some anions did not behave as expected. In sum, these properties of secreting chromaffin cells resembled some properties of isolated chromaffin granules incubated in ATP and Cl^-, but were different in a number of instances. Therefore, we have interpreted our data to indicate that while some mechanistic relationships may indeed exist between the release event in exocytosis from chromaffin cells and the chemiosmotic lysis reaction chracteristic of isolated chromaffin granules, an understanding of the energetics of exocytosis awaits the discovery of reasons for the quantitative differences between the two systems. On the other hand, pancreatic islets of Langerhans were found to obey the chemiosmotic predictions almost exactly.

Work has also been progressing on the problem of how the membrane contact and fusion mechanism of exocytosis may occur in our studies on synexin. Synexin is a 47,000 dalton protein from adrenal medulla and other tissues which can be activated by calcium to bind to chromaffin granules. Upon binding to synexin, these granules aggregate with one another by forming multiple pentalaminar membrane contacts. Addition of small amounts of arachidonic acid to the aggregated granules cause them to fuse, forming large vacuolar structures, reminiscent of the compound exocytotic structures found in secreting cells.

In the Section on Membrane Biology, the emphasis has been on mechanisms of phagocytosis and complement action. In some ways, these events are the exact opposite of exocytosis and studies of both processes ought to be viewed as complementary. Mutants from a macrophage cell line have been isolated that are deficient in general phagocytosis. This is achieved by selecting, after mutagenesis, cells that were not killed by exposure to toxin-derivitized plastic beads. Several cell lines thus selected proved resistant because they could no longer phagocytose plastic beads, and not because of resistance to the toxin.

Differences in virulence among three Salmonella strains have been found to be due to differential complement activation by the bacteria. It has now been found that this activation, via the alternative pathway, is due solely to the differences in lipopolysaccharide (LPS) structure of the three strains. We postulate that the O-antigen polysaccharide of LPS is an important virulence determinent in these Salmonellae: if the LPS activates complement, the resultant C3b-coated bacterium is phagocytosed by macrophages and possibly destroyed; if the LPS structure is not an activator, the bacterium can escape ingestion and destruction. Current experiments are intended to determine exactly which enzymatic reaction is affected by the structure of the O-antigen of LPS. We have also been interested in how the terminal attack complex of complement (C5b-9) exerts its effects. It is known that the O-antigen on all of the strains prevents killing by the terminal attack complex of complement (C5b-9). Strains lacking O-antigen ("rough" strains) are killed, and previous experiments suggest that this killing was caused by ability of the C5b-9 complex to approach and insert into the membrane, while O-antigen chains might sterically hinder its access. We now know that the sensitivity to killing changes abruptly when the density and/or length of the O-antigen-bearing LPS molecules falls below or rises above a certain level.

The Section on Cytogenetics has been particularly interested in the inheritance of autoimmune traits, cytogenetics of disease, and bone marrow transplantation. The proliferative capacity of splenic and peritoneal B cells from NZB/N female mice cultured in the presence of BCGF has been studied. In normal animals, only substantial proliferative responses were obtained when B cells were cultured in the presence of anti-IgM and BCGF. However, B cells from autoimmune animals reached similar proliferation in response to BCGF and anti-IgM or BCGF alone, meaning that autoimmune B cells are in a pre-activated state. T cells can be activated in vitro by incubation with concanaralin A, a T cell mitogen, and phorbol myristate acetate (PMA), a co-mitogen. Autoimmune MRL/lpr T cells are incapable of responding to con A either in terms of proliferation or production of the lymphokine IL-2. However, the co-mitogen PMA was able to restore the proliferative capacity to cells stimulated with con A, but was not able to restore normal IL-2 production. These studies demonstrate a dissociation between IL-2 production and T cell proliferation.

Stem cell activity has also been considered as a possible underlying basis for autoimmune syndromes. The response to two types of stem cell signals was studied in DBA/2 and in autoimmune NZB mice: a specific erythroid stem cell signal following bleeding, and a non-specific stem cell differentiation signal following repopulation of lethally irradiated (NZB X DBA/2)F1 NZB mice manifest differential early stem cell proliferation: normal erythroid but increased lymphoid. The abnormal lymphoid stem cell regulation may thus underlie their autoimmune syndrome.

Studies of T cell function in rejection of transplants such as bone marrow have also been performed. Advantage has been taken of the fact that approximately 25% male donor takes were observed cytogenetically in recipient female spleens and marrows, while only 5% female cells were found in male recipients. The effect was not hormonal since these studies demonstrated that the rejection of female cells in transfused male mice was immunologically mediated and dependent on the presence of T cells from the donor female mice.

We have also begun to evaluate types of cells which repopulate irradiated animals. This study was performed using fluorescently-tagged, monoclonal antibodies which recognize specific subsets of cells of lymphoid or erythroid origin. The fluorescence was detected by using a fluorescence activated cell sorter. It was found that mature cells were initially B lymphocytes, whereas in animals repopulated with normal bone marrow cells, mature lymphoid cells constituted less than 30% of the total cells at two weeks. By two months post-irradiation and repopulation, no difference was observed between autoimmune and normal stem cell differentiation. Thus, NZB marrow stem cells more readily differentiate into B lymphocytes than normal stem cells. This study has been completed and a manuscript submitted. We have also continued our studies of the kinetics of hemopoietic stem cells by means of marrow transfusions. It may be recalled that the seeding of transfused marrow cells has long been thought to require special proliferative sites.

Using the combination of cytogenetic and electrophoretic techniques we have investigated (1) the deleterious effect of transfusion itself on marrow cells and (2) the effects of exposure of marrow cells to an irradiated environment when the marrow cells were not exposed to irradiation. The results are that normal marrows transfused serially into irradiated hosts become "exhausted." We now believe that transfusion itself may be the cause of marrow being no longer able to restore lethally irradiated mice after 4 to 5 passages. The techniques we have developed will make it possible to verify or reject that hypothesis.

The Section on Macromolecular Genetics continues to employ DNA viruses such as adeno-associated virus as molecular probes to study gene expression in human cells.

We have constructed bacterial plasmids (e.g., pAV2 containing the entire 4.7 kb DNA genome of the human parvovirus, adeno-associated virus type 2). Because the AAV genome was inserted into the plasmid DNA using *BglII* linkers, the entire virus genome can be recovered by *in vitro* cleavage of the purified recombinant plasmid. These AAV-plasmid recombinant DNA molecules are useful both for site-specific mutagenesis of the viral genome and to study the potential of AAV as a eukaryotic vector. Continued passage of the human parvovirus, adenoassociated virus (AAV), at high multiplicity of infection in human cells results in the accumulation of AAV particles containing variant genomes. Infectious AAV genomes were also generated by recombination when cells were jointly transfected with a mixture of plasmids containing two different mutant AAV genomes. The efficiency of this recombination appear to be influenced by the degree of homology between the mutant AAV genomes. This may be useful for growth of pure populations of mutant AAV particles. The pBR322 based recombinant plasmid DNA, pAV2, containing the entire adeno-associated virus (AAV) type 2 genome has been used to construct *in vitro* mutants, containing deletions within the AAV sequence. Analysis of these deletions has shown that there may be an additional region of AAV, perhaps within the intron, which is required for efficient particle assembly. This work also shows that AAV is genetically complex and expresses at least three clearly different functions. AAV has also been developed as a novel eukaryotic vector (*parvector*) for the expression of foreign genes in human cells. The vector, pAV2, contains the entire AAV genome in a pBR322-derived bacterial plasmid. We inserted a prokaryotic sequence coding for chloramphenicol acetyl transferase (CAT) into pAV2 following either of the AAV promoters. Both AAV vectors could be packaged into AAV particles by complementation with wild type AAV and yielded CAT activity when subsequently infected into cells in the presence of adenovirus. These studies thus showed that AAV can be used as a eukaryotic expression vector.

In separate studies on Herpes simplex virus, a variety of DNA clones of HSV DNA in both plasmid and phage vectors have been established. Two potential shuttle vectors, each containing the identical Herpes simplex virus type I (HSV-1) defective particle DNA (dDNA), but prokaryotic DNA of different origin, were examined for their stability when propagated in eukaryotic cells, and for their efficiency as shuttle vectors. These results suggest additional considerations when designing single units of HSV-1 dDNA for use as vectors to accomodate large fragments of DNA and specifically that large inserts of unselected sequence may be unstable in HSV-1 DNA derived vectors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM21,001-30 LCBG

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Preparation of stained tissue sections for investigation and diagnostic purposes.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Virginia Reveal Head, Tissue Preparation Lab. LCBG:NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Cell Biology and Genetics

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NIADDK:NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.3	0	0.3

CHECK APPROPRIATE BOX(ES)

_ (a) Human subjects _ (b) Human tissues XX_ (c) Neither

_ (a1) Minors

_ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Inactive.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM21,002-19 LCBG

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cellular Function and Ultrastructure

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Lois W. Tice	Chief, Section on Cellular Function and Ultrastructure	LCBG	NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Cell Biology and Genetics

SECTION
Cellular Function and Ultrastructure

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0	0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Terminated

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM21,003-16 LCBG

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biomechanics and related subjects

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: C.W. McCutchen Research Physicist LCBG:NIADDK

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Cell Biology and Genetics

SECTION
Cell Biology and Biochemistry

INSTITUTE AND LOCATION
NIADDK:NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

No work was completed during the year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 21,005-17 LCBG

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Histochemistry: Principles, Methods and Applications

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	N. Feder	Medical Officer(Research)	LCBG, NIADDK
Others:	W. Stewart	Research Physicist	LCBG, NIADDK
	L. Knipling	Biologist	LCBG, NIADDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Cell Biology and Genetics

SECTION

Section on Biophysical Histology

INSTITUTE AND LOCATION

NIH, NIADDK, Bethesda, Md.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Continuing studies with the dye, Lucifer Yellow, have been carried out.

Studies have continued on the genetics of nerve cell shape using the snail Biomphalaria glabrata as an experimental animal.

Studies are nearing completion on the professional practices of a group of biomedical scientists.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 21,008-18 LCBG

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cytogenetics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.H. Tjio	Chief, Section on Cytogenetics	LCBG, NIADDK
Others:	B.J. White	Medical Officer (Research)	LCBG, NIADDK
	E.S. Raveche	Research Chemist	LCBG, NIADDK

COOPERATING UNITS *(if any)*
Arthritis and Rheumatism Branch, NIADDK, NIH (A. Steinberg); University of California, Berkeley (C. Brecher).

LAB/BRANCH
Laboratory of Cell Biology and Genetics

SECTION
Cytogenetics

INSTITUTE AND LOCATION
NIH, NIADDK, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2	1	1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1. Application of differential staining techniques and continued improvement of established methods are basic to all of the projects conducted in the laboratory.

2. Human cytogenetic studies of patients with congenital and developmental disorders as well as studies of mutagen-induced chromosomal abnormalities are continuing.

3. Murine cytogenetic studies include evaluation of mitotic and meiotic chromosomes and karyotypic evaluation of cells in hematopoetic tissue regeneration experiments. These are part of projects utilizing the mouse as a model for human diseases.

4. Correlation of flow cytometric analysis with cytogenetic findings.

5. Study of the fate of opposite sex syngeneic transfers in non-irradiated mice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM21,009 -17 LCBG

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cytogenetic studies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	White, B.J.	Research Medical Officer	LCBG:NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Cell Biology and Genetics

SECTION

Cytogenetics

INSTITUTE AND LOCATION

NIADDK:NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Inactive - Dr. White on sabbatical to the University of Washington.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM21,010-08 LCBG

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Function of DNA virus genomes in animal cells.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Barrie J. Carter	Chief, Section on Macromolecular Genetics	LCBG:NIADDK
Others:	Periannan Senapathy	Visiting Associate	LCBG:NIADDK
	Michael H.P. West	Visiting Associate	LCBG:NIADDK
	Jon-Duri Tratschin	Visiting Associate	LCBG:NIADDK
	Irving Miller	Biologist	LCBG:NIADDK
	Ehud Katz	Visiting Scientist	LCBG:NIADDK

COOPERATING UNITS *(if any)*

L. Enquist, R. Watson, Molecular Genetics Inc., Minnesota, S. Bear, D. Court, NCI

LAB/BRANCH

Laboratory of Cell Biology and Genetics

SECTION

Macromolecular Genetics

INSTITUTE AND LOCATION

NIHADDK:NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
6.0	5.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are employing DNA viruses as molecular probes to study genome expression in human cells. We are studying intensively the structure and function of adeno-associated virus (AAV) since this virus has only a small genome. AAV has also been developed as a eukaryotic expression vector. We are studying also adenovirus which is a more complex genome. Adenovirus is the helper virus for AAV multiplication. This helper relationship is being analysed. Also both AAV and adenovirus recombine with cellular DNA. In the case of adenovirus this causes malignant transformation of the cell. AAV inhibits this transformation and also inhibits Ad12 oncogenesis in newborn animals. Thus AAV inhibits tumor induction. The mechanism of this inhibition of tumor induction is being studied at the molecular level in both cell culture and in animal experiments. We are also analysing gene expression and recombination in herpes simplex virus which is also a helper for AAV and has been linked to cervical carcinoma.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 21,012-07 LCBG

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cytogenetics and Immunogenetics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E.S. Raveche	Research Chemist	LCBG, NIADDK
Other:	J.H. Tjio	Chief, Section on Cytogenetics	LCBG, NIADDK

COOPERATING UNITS *(if any)* Arthritis and Rheumatism Branch, NIADDK, NIH (A. Steinberg); Laboratory of Microbial Immunity, NIAID, NIH (T. Chused); Clinica Puerta de Hierro, Madrid, Spain (C. Gutierrez); Food and Drug Administration (P. Noguchi).

LAB/BRANCH
Laboratory of Cell Biology and Genetics

SECTION
Cytogenetics

INSTITUTE AND LOCATION
NIH, NIADDK, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 2	PROFESSIONAL: 2	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1. Application of differential staining techniques for chromosome analysis.

2. Studies of the inheritance of several autoimmune immunological traits including antibodies cytotoxic for T lymphocytes and reactive with single stranded DNA and chromosomal hyperdiploidy.

3. Use of flow cytometry in the analysis of aneuploid cells.

4. Analysis of radiation chimeras to determine if autoimmune stem cells have increased repopulation potential.

5. In vivo cell cycle analysis of polyclonal B-cell activation by flow cytometric techniques.

6. The use of recombinant inbred lines to determine the presence of an underlying defect - giving rise to autoimmune disease.

7. Syngeneic bone marrow transfers to study fate of transferred marrow.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM21,018-02 LCBG

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of membranes in macrophages and in bacteria

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Loretta Leive	Chief, Section on Membrane Biology	LCBG:NIADDK
Others:	Nili Grossman	Visiting Fellow	LCBG:NIADDK
	Fronz Emling	Guest Worker	LCBG:NIADDK
	Loretta Cullinane	Biologist	LCBG:NIADDK
	Emelie Klima	Chemist	LCBG:NIADDK

COOPERATING UNITS *(if any)*

Dr. Michael Frank, LCI:NIAID; Dr. Keith Joiner, LCI:NIAID; Dr. Benes Trus, Computer System Lab., DCRT.

LAB/BRANCH

Laboratory of Cell Biology and Genetics

SECTION

Membrane Biology

INSTITUTE AND LOCATION

NIADDK:NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We previously isolated cells impaired in general phagocytosis from a macrophage cell line by selecting mutants resistant to killing by toxin-derivitized plastic beads. Several mutants could not phagocytose beads, but were normal in receptor-mediated phagocytosis. We now find that two mutants, unlike the parent, are detached from flasks by trypsin. The defects in the mutants are under study.

Differences in virulence among three Salmonella strains were shown by us to result from differential complement (C) activation via the alternative pathway. Only the structure of O-antigen (O-Ag) in their lipopolysaccharide (LPS) differs among these strains; this difference causes the different C activation since the results were mimicked by purified LPS coated on sheep erythrocytes. We now find that during activation, C3b deposition on either bacteria or LPS-coated erythrocytes is proportional to C3 activation, and that the degradation of this surface-bound C3b (e.g., to iC3b) is independent of LPS structure. Thus, slight differences in O-Ag composition affect C3 activation but not its degradation; the resultant covalently bound C3 fragments presumably determine subsequent phagocytosis by macrophages via the approporate receptors, thereby influencing bacterial virulence.

Bacteria containing O-Ag are not killed by the terminal attack complex of C (C5b-9) but those lacking O-Ag are. Using a Salmonella mutant in which the synthesis of O-Ag can be varied, we found that sensitivity to killing increased abruptly when < 60% of the LPS molecules have O-Ag, and < 20% of the LPS molecules have long (15-55 repeating units) O-Ag chains. Further, two classes of E. coli mutants resistant to C killing were both found to have increased amounts of LPS; one also had increased substitution of LPS with O-Ag. These results, with different organisms, indicate that a specific critical degree of steric hindrance by O-Ag prevents access of the C5b-9 complex to the membrane.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM21,019-02 LCBG

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanism of hormone and transmitter secretion.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)*

P.I.: Harvey B. Pollard Chief, Laboratory of Cell Biology and Genetics NIADDK

Others: Richard Ornberg, Ph.D. SSF; George Lee, Ph.D. Res. Chemist; Eduardo Rojas Ph.D., VS; Illani Atwater, Ph.D., Expert; Rosa Santos, VA; Mark Levine, M.D. RA; Katrina Kelner, Ph.D. Prat; Robert Levine, Ph.D. Prat; Keith Brocklehurst, Ph.D. GW; Patrick Flemming, Ph.D. GW; Le Duong, Ph.D. SF; Kyoji Morita, Ph.D. VA; Moussa Youdim, Ph.D. VS; Peter Lelkes, Ph.D. VA; Eli Heldman, Ph.D., VS; Luis Rosario, Ph.D. VF; Phillipe Lebrun, M.D. VA; Artur Aguas, M.D. VF; Avner Ramu, M.D. VS; Erik Forsberg, Ph.D. SF.

COOPERATING UNITS *(if any)*

Dipak Banerjee, Ph.D., NIDR; David Rodbard, M.D., NICHD; Walter Lovenberg, Ph.D., NIHLB.

LAB/BRANCH

Laboratory of Cell Biology and Genetics

SECTION

Cell Biology and Biochemistry

INSTITUTE AND LOCATION

NIADDK:NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
21.5	21.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our recent studies have focussed on the processes underlying granule assembly, synthesis and insertion of catecholamines into granules, movement of granules to sites of secretion and membrane contact and fusion between granules and plasma membranes leading to exocytosis in chromaffin cells. Some work has also been done with Islets of Langerhans. Our work this year has included the synthesis and packaging of vasoactive intestinal peptide, as well as synthesis of norepinephrine from transported dopamine within the granule. Ascorbic acid seems to act on the enzyme dopamine-β-hydroxylase from outside the granule. A mystery concerning how catecholamines can be synthesized in such quantity in chromaffin cells, in the face of massive monoamine oxidase activity has been solved. The MAO in chromaffin cells is type B, with low affinity for β-hydroylated catecholamines. The membrane contact and fusion event, thought to be involved with calcium and synexin, is now more fully understood, by both fast freezing fixation and electron microscopy, as well as by monodonal antibodies directed against synexin. The pharmacology of insulin secretion from Islets of Langerhans also seems to follow the pharmacology of synexin, not calmodulin as hitherto thought. Finally, we were able to isolate the neuroendocrine barrier of the adrenal medulla, the endothelial cells making up the capillary network supporting the endocrine chromaffin cells. These cells were found to form true capillaries in vitro, and constitute an exciting new thrust forward in our attempts to understand adrenal gland endocrinology.

ANNUAL REPORT OF THE LABORATORY OF BIOCHEMICAL PHARMACOLOGY
NATIONAL INSTITUTE OF ARTHRITIS, DIABETES, AND DIGESTIVE AND KIDNEY DISEASES

I. POLYAMINES

Polyamine Biosynthesis in Escherichia coli. Our overall studies have been concerned with the biosynthesis, metabolism, and function of the polyamines, particularly putrescine and spermidine. In this part of the work we have concentrated on the biosynthesis and function of polyamines in *Escherichia coli*. We have continued our studies on the specific genes and enzymatic steps involved in the biosynthesis of these amines. Mutants in these genes have been selected, and recombinant DNA plasmids have been constructed. Strains containing these plasmids have been used for the overproduction of the respective enzymes.

During the past year we have been particularly interested in the structure and mechanism of one of the enzymes involved in spermidine biosynthesis, namely, adenosylmethionine decarboxylase, which is coded for by the *speD* gene. We had previously shown (with Dr. R. B. Wickner, LBP) that this enzyme contains covalently-linked pyruvate, and this moiety is required for activity; we are now studying the mechanism of formation of this unusual protein component. As part of this study the *speD* gene and the gene product (adenosylmethionine decarboxylase) are being sequenced, and better recombinant plasmids have been constructed for the overproduction of the enzyme. Antibodies have been prepared for the detection of the enzyme and any proenzyme in minicell preparations.

We have also been continuing our studies on the physiological role of these amines, using our mutants that lack all of the biosynthetic pathways. We have shown that polyamines affect the conformation or stability of the protei synthesizing ribosomal complex *in vivo*. Studies have continued on the effect of polyamines on the growth of various bacteriophages and on their role in permitting the readthrough of amber mutants.

. . . . . Drs. H. Tabor and C. W. Tabor

Polyamine Biosynthesis in Yeast. We have continued our studies on the biosynthesis, regulation, and function of polyamines in *Saccharomyces cerevisiae*. Our major effort during the past year has been on studying ornithine decarboxylase, the first step in the biosynthetic pathway for polyamines in yeast. We are studying the mechanisms of control in this system as a model for the eukaryotic enzyme, which has been of special interest because it increases very rapidly under conditions of rapid growth and differentiation.

We have been studying five problems related to this enzyme: (1) purification of the enzyme; (2) regulatory control of enzymatic activity; (3) genetics of the enzyme; (4) development of methods to facilitate isolation of clones carrying the gene; and (5) physiologic effects of polyamine deprivation.

(1) *Purification Procedure*: Procedures have been developed for the purification of a stable form of an 86,000 Mr protein, which is secondarily converted by proteolytic enzymes to a 68,000 Mr and a 35,000 Mr form. The enzyme has been shown to be a glycoprotein.

(2) The enzyme loses activity after growth in the presence of polyamines; no obvious protein degradation is involved and the regulation is by a putative post-translational modification.

(3) We have shown that all of the *spe10* mutants, which lack ornithine decarboxylase activity, are located in the same genetic locus. It is still uncertain if this defect is in a regulatory or a structural gene.

(4) A screening technique has been developed to select for strains that might have a recombinant plasmid carrying the gene for ornithine decarboxylase (or other genes in the polyamine pathway). Banks of recombinant plasmids have been constructed.

(5) We have also been using the various mutants that we previously described to study the physiologic effect of polyamine deprivation. We have found that amine-deficient cells arrest in a budded stage, similar to cell division cycle mutants.

. . . . . Drs. C. W. Tabor, H. Tabor, and S. K. Taneja

II. MECHANISMS OF INHERITANCE IN *SACCHAROMYCES CEREVISIAE*

Background. Yeast carries up to five different dsRNAs, called L-A, L-(BC), T, W, and M. Killer strains of yeast secrete a protein toxin to which they are themselves immune. Toxin and immunity are determined by the M dsRNAs: M_1 (1.5 kb) for the K_1 system and M_2 (1.0 kb) for the K_2 system. The replication of M_1 or M_2 depends on L-A (4.5 kb), which encodes the major protein of the virus-like particles in which L-A and M dsRNAs are found. Thirty chromosomal genes (*MAK*) are necessary for M_1 or M_2 replication. Two genes (*MKT*) are specifically needed for M_2 and only if L-A-HN is present.

Genetic Control of L-A and L-(BC) Copy Number. We have shown that M dsRNA represses L-A replication but does not affect L-(BC), while the six chromosomal *SKI*$^+$ gene products repress L-A, L-(BC), and M dsRNA replication, as shown by 3- to 10-fold lowering of their copy numbers. The effect of M on L-A is epistatic to that of *SKI* products. We have isolated a *ski*$^-$ strain that can maintain M_1 in spite of its lacking L-A completely.

. . . . . Dr. S. G. Ball, C. Tirtiaux, and Dr. R. B. Wickner

Topoisomerases and dsRNA Replication. *MAK1* has been shown by others to be the gene for yeast topoisomerase I. Our work showed this gene's involvement in M dsRNA replication (but not L-A, L-B, T, or W). We recently found that all three mutant alleles of *MAK1* confer cold sensitivity for cell growth (8°C), although growth is normal from 20°C to 37°C.

The gene for yeast topoisomerase II was located by others near *MET4* on chromosome XIV. We recently mapped the *MKT1* gene on chromosome XIV, also near *MET4*, and are investigating the possible identity of these two genes. They are at least very close to each other, as no recombination was found in a total of 41 meiotic tetrads.

One allele of MKT1, when combined with a particular cytoplasmic element, result in cold sensitivity for cell growth (8°C). Preliminary evidence suggests tha the cytoplasmic element is L-(BC).

. . . . . Drs. R. B. Wickner and S. S. Sommer

Molecular Cloning of SKI$^-$ Genes and Their Suppressors. We previously showed that ski$^-$ mutants carrying M dsRNA are cold-sensitive for growth because their M copy number is elevated. Using the cold sensitivity of ski$^-$ strains carrying M dsRNA, we have cloned segments of yeast DNA that suppress this effect. These should include the SKI$^+$ genes themselves and genes, overproduction of whose products suppress this phenotype.

. . . . . Drs. S. S. Sommer and R. B. Wickner

T and W dsRNAs. We have discovered a chromosomal gene affecting the copy number of both T and W dsRNAs.

. . . . . Dr. R. B. Wickner

III. NUCLEIC ACIDS

All metazoans examined to date contain families of repetitive DNA sequences, and suggestions for their function include coordination of the expression of tissue-specific genes, promotion of chromosomal rearrangements such as duplications, inversions, and deletions, a role in speciation, or even no function except self-propagation as "selfish" DNA. We have developed an experimental approach to rapidly characterize and identify rat repeated DNA sequences of potenti interest, and in our earlier studies we identified a number of highly repeate transcribed DNA sequences that appear to be organized as permuted clusters. In examining the genomic regions that contain these sequences we found that one class of them is located in long, 3-7 kb structures that are themselves repeated about 50,000 times in the rat genome. These structures are the counterparts in rats of long interspersed repeated elements (referred to as LINES) that have been identified in mice and primates. We have determined their overall structure and found that, although they are quite polymorphic, they share common architectural features as revealed by the spacing of certain restriction enzymes sites. In addition we have shown that rat LINES appear to be mobile in the rat genome since they produce heterozygosity among individual rats of different laboratory strains as well as of wild rats. Three such loci are the murine leukemia virus integration site 2, the immunoglobulin heavy chain locus, and the rat insulin I locus. The latter case is particularly intriguing because amplification of the rat insulin I locus and flanking DNA accompanies the presence of the rat LINE.

We also found that rat repeated sequences can be classified as either "G:C-rich" or "A:T-rich". The latter are the ones that comprise rat LINES, and genomic regions that contain LINES contain few, if any, of the G:C-rich sequences. On the other hand, the regions of the genome that contain multiple copies of the G:C-rich sequences appear to lack LINES. In an attempt to confirm the impression that G:C-rich and A:T-rich repeated sequences are segregated in the genome, we are using *in situ* hybridization to map the G:C-rich and A:T-rich repeated sequences at the chromosomal level.

. . . . . Drs. A. V. Furano, E. D'Ambrosio, and M. S. Lakshmikumaran, and A. Lo Cuoco and A. Salemme

DNA Replication: The Bacteriophage T4 System. We are studying the *Escherichia coli* bacteriophage T4 as a model system for duplex DNA replication. Efficient DNA synthesis *in vitro* can be achieved by a mixture of seven proteins encoded by T4 phage: gene 43 T4 DNA polymerase, gene 32 DNA helix destabilizing protein, gene 44/62 and 45 polymerase accessory proteins, and gene 41 and 61 priming proteins.

Cloning and Expression of the Genes Encoding the T4 Priming Proteins. The products of both genes 41 and 61 are required to synthesize the pentaribonucleotides that initiate new DNA chains on the lagging strand of a replication fork. The 41 protein also has a DNA unwinding (helicase) activity that increases the rate of DNA synthesis. Since our studies of the priming and helicase reactions have been hampered by the low level of these proteins in the infected cell, we have cloned each of the priming protein genes in multicopy pBR322-derived plasmids. Cells with plasmids in which gene 41 (T4 map units 20.1 to 22.0) is downstream of the phage λ P_L promoter produce 100-fold more 41 protein than is present in an infected cell. We have located gene 61 downstream of gene 41 between T4 map units 15.0 and 18.7. This location is about 1,000 bp downstream of two strong promoters located by *in vitro* transcription of appropriate T4 DNA restriction fragments. Partially purified fractions from cells with plasmids which include this region contain gene 61 priming activity at levels close to that in a T4 infected cell.

Identification of a New T4 Double-Stranded DNA Binding Protein. Plasmids containing T4 DNA upstream of gene 41 produce a 46 kd protein that binds tightly to double-stranded DNA. A protein of the same size is made by *in vitro* transcription-translation of DNA from these plasmids or T4 DNA restriction fragments from the same map region. Based on the truncated protein produced by a plasmid with an 800 bp deletion, we position the 46 kd protein gene between 22.2 to 23.7 map units, which is about 500 bp downstream of two strong promoters detected *in vitro*. The 46 kd protein appears to be encoded or in some way controlled by the T4 *uvsX* gene, whose product is required for DNA recombination and repair of UV damage. The T4 *fdsA* mutant in the *uvsX* gene has been shown by Dewey and Frankel to suppress T4 mutants in the gene *49* nuclease, apparently because the recombination-deficient phage produce fewer branched DNA molecules that are normally cut by the 49 nuclease. We find that this suppression does not occur in cells with plasmids expressing the 46 kd protein, although it occurs normally in control cells containing plasmids with the 800 bp deletion. Moreover, the 46 kd double-stranded DNA binding protein which we find in extracts of T4 infected cells is not present after infection with the *fdsA* or *am* 11 mutants in the *uvsX* gene. Previous studies in other laboratories have assigned the *uvsX* gene to a 38-40 kd *recA*-like protein that promotes homologous strand exchange *in vitro*. The relationship between this smaller protein and our 46 kd protein is not yet clear. If they are indeed products of the same DNA, one protein could be processed from the other or the two could be encoded by overlapping genes.

. . . . . Drs. D. M. Hinton and N. G. Nossal

Initiation of T4 DNA Replication. We are continuing our efforts to develop a *in vitro* system to study the initiation of T4 DNA replication. We have fou conditions under which fractions from T4 infected cells show a preference for T4 DNA over other double-stranded linear DNA. Even at the earliest times the DNA product hybridizes equally well to DNA from all regions of the T4 genome. Synthesis is markedly decreased in fractions from cells infected with phage lacking the gene 41 or gene 45 replication proteins.

. . . . . Dr. N. G. Nossal

The presence of DNA was demonstrated in the sera of patients with chronic non-A, non-B hepatitis and from chimpanzees experimentally infected with the non-A, non-B patient's serum. An *EcoRI* fragment of the non-A, non-B serum DNA has been cloned into pBR322 (e.g., *a* 780 bp fragment). Hybridization with serum DNA of non-A, non-B infected chimpanzees and the 780 bp *EcoRI* fragment was observed only during peak alanine aminotransferase elevation. Reverse transcriptase activity can be pelleted from non-A, non-B sera by polyethylene glycol 6000 and two high speed centrifugation methods. cDNA has been prepared from reverse transcriptase positive pellets resulting from centrifugation, and cloning of this cDNA is in progress.

. . . . . Dr. W. G. Coleman, Jr., and Drs. B. P. Seto and R. J. Gerety (DBBP, NCDB, FDA)

Evolution. Hydrogen bonding between the purines, adenine and guanine, and the tripeptide, triglycine, has been demonstrated. Binding in aqueous solution between the peptide Lys-Ser-Gly-Lys and a newly synthesized 10-member oligonucleotide of cytosine has been shown by measuring the circular dichroi spectrum of (dC)10.

. . . . . Drs. C.-H. Niu and S. Black

IV. COMPLEX CARBOHYDRATES

Cell surface carbohydrates change during development as they are probably involved in differentiation. These developmentally-regulated changes allow some antibodies directed against carbohydrates to discriminate among tissues, both normal and malignant. To obtain monoclonal antibodies specific for various cell types, mice and rats have been immunized with human tissues in many laboratories. Some of the antibodies derived from spleen cells of the immunized animals have an apparent specificity for certain cancers. Out of 460 of these antibodies sent to us from several laboratories in the past 4 years, 121 are directed against carbohydrate sequences in glycoproteins and glycolipids as determined by solid-phase radioimmunoassay and by immunostaining of thin layer chromatograms of glycolipids. Of the 121 antibodies, 23 are directed against human blood group antigens including 7 against the *H* type 1 or H type 2 antigens, 4 against the Le^b antigen, 1 against the Le^a antigen, 8 against the A antigen, and 3 against the B antigen. Fifty-six antibodies are directed against a sugar sequence found in the human milk oligosaccharide lacto-*N*-fucopentaose III, 1 against a sugar sequence found in the human milk oligosaccharide lacto-*N*-neotetraose, 2 against a sialylated Le^a antigen, and 40 against unidentified carbohydrate sequences in glycolipids and/or glycoproteins.

. . . . . Drs. V. Ginsburg, P. L. Fredman, J. L. Magnani, D. D. Roberts, P. F. Spitalnik, and S. L. Spitalnik

V. MEMBRANE STUDIES OF ESCHERICHIA COLI

Previously, a novobiocin-hypersensitive mutant of Escherichia coli K-12 carrying a cysE-pyrE linked mutation, designated rfaD, which specifically affects the synthesis of the aldoheptose, L-glycero-D-mannoheptose, has been isolated and genetically characterized. The following facts have been established: (1) The truncated lipopolysaccharide (LPS) of the rfaD mutant contains D-glycero-D-mannoheptose rather than the normal L-glycero-D-mannoheptose. (2) The rfaD gene product, ADP-L-glycero-D-mannoheptose-6-epimerase, is required for the conversion of ADP-D-glycero-D-mannoheptose to ADP-L-glycero-D-mannoheptose. (3) The nucleotide ADP-D-glycero-D-mannoheptose accumulates in the rfaD strains. These results provide support for the following biosynthetic reactions: (a) the conversion of D-glycero-D-mannoheptose-1-P and ATP to ADP-D-glycero-D-mannoheptose and pyrophosphate and (b) ADP-D-glycero-D-mannoheptose is converted to ADP-L-glycero-D-mannoheptose. These two reactions are catalyzed by the enzymes ADP-D-glycero-D-mannoheptose-synthetase and ADP-L-glycero-D-mannoheptose-6-epimerase, respectively.

The rfaD gene has been cloned into pBR322, and the rfaD gene product has been partially purified from plasmid amplified strains.

Recently, we have isolated a new mutant carrying a cysE-pyrE linked mutation, designated rfa-2, which results in an increased permeability to hydrophobic agents and a heptose-less LPS structure. Further, we have demonstrated that the rfa-2 mutation is genetically distinct from the rfaD locus and the rfa-2 phenotype is not abolished by plasmid carrying the wild type rfaD allele. The rfaD gene product, ADP-L-glycero-D-mannoheptose-6-epimerase, is detectable in crude extracts of rfa-2 mutant strains. An automated sugar analyzer has been developed in our laboratory which allows us to follow directly the conversion of the sugar intermediates involved in the biosynthesis of L-glycero-D-mannoheptose.

. . . . . Dr. W. G. Coleman, Jr.

VI. ENZYME MECHANISMS AND PROTEIN STRUCTURE

The α and β_2 subunits and the $\alpha_2\beta_2$ complex of tryptophan synthase from three sources (Escherichia coli, Salmonella typhimurium, and an interspecies hybrid) have been purified and characterized with the aim of understanding the effects of the divergent evolution of E. coli and S. typhimurium upon the structure and function of enzymes. Studies of limited proteolysis of the α and β_2 subunits have demonstrated several sites of proteolysis. We have discovered that the amino-terminus of the β_2 subunit is incompletely processed when the enzyme is produced at a high level.

. . . . . Drs. E. W. Miles, S. A. Ahmed, T. Fairwell (MD, NHLBI), K. Yutani (Institute for Protein Research, Osaka University, Japan), and R. Bauerle (University of Virginia, Charlottesville)

The first crystals of the $\alpha_2\beta_2$ complex of tryptophan synthase suitable for X-ra diffraction studies have been grown from the enzyme from *S. typhimurium* Preliminary X-ray crystallographic data have established the space group and unit cell dimensions and have found one αβ pair of MW = 76,000 within the asymmetric unit. The crystals appear suitable for a complete structure investigation.

. . . . . Drs. E. W. Miles and S. A. Ahmed and Dr. D. R. Davies (LMB, NIADDK)

Our finding that two tryptophan analogs, indolyl-L-alanine and 2,3-dihydro-L-tryptophan, are potent competititive inhibitors of both tryptophan synthase and tryptophanase is evidence that the indolenine tautomer of L-tryptophan, which resembles the inhibitors in its geometry at C-3 of the heterocyclic ring, is an intermediate in the reactions catalyzed by both enzymes. Another L-tryptophan analog, 6-azido-L-tryptophan, is an effective photoaffinity inhibitor.

. . . . . Dr. E. W. Miles and Drs. R. A. Phillips and L. A. Cohen (LC, NIADDK)

We have discovered that phenylglyoxal and p-hydroxyphenylglyoxal, which are generally considered arginine-specific reagents, also react with certain protein sulfhydryl residues and with a model sulfhydryl compound. Phenylglyoxal modifies three essential residues in the α subunit of tryptophan synthase: two sulfhydryl groups and one arginine. The substrate indole 3-glycerol phosphate protects the essential arginine from modification.

. . . . . Drs. E. W. Miles and H.-M. Eun

Studies of the substrate and reaction specificity of tryptophan synthase show that the enzyme can synthesize a number of S-substituted L-cysteine and S-substituted β-methylcysteine derivatives by β-replacement reactions. Se-substituted L-selenocysteines can be synthesized from L-serine and selenols by the β-replacement reactions catalyzed by tryptophan synthase.

. . . . . Dr. E. W. Miles and Drs. N. Esaki, H. Tanaka, and K. Soda (Institute for Chemical Research, Kyoto University, Japan)

Data taken from the literature on receptor-mediated stimulation of adenylate cyclase have been reviewed and analyzed to determine whether and to what extent these data are compatible with several previously proposed mechanistic molecular models and extensions thereof.

. . . . . Dr. A. P. Minton

The kinetics of appearance of fibrinopeptides following the addition of thrombin or other specific enzymes to fibrinogen are being measured via amino acid analysis of fibrinopeptide hydrolyzates.

The kinetics of appearance of small soluble oligomers of fibrinogen/fibrin at early times in the fibrin gelation reaction are being measured via a novel application of high performance gel exclusion chromatography.

. . . . . Drs. J. Wilf and A. P. Minton

The dependence of fibrin gelation time upon initial concentrations of fibrinogen and thrombin, upon the delay time preceding addition of a thrombin inhibitor, and upon the concentration of unrelated globular proteins present in the reaction mixture, is being measured using a novel viscometric technique.

. . . . . Drs. J. Wilf, J. A. Gladner, and A. P. Minton

An automated method is being developed for measuring the concentration gradients of individual macromolecular species in a centrifuged solution containing several macromolecular solutes.

. . . . . Drs. A. K. Attri and A. P. Minton

Protein Synthesis. The activation of a valyl-tRNA synthetase discovered in this laboratory has been found to be powerfully inhibited by unsaturated fatty acids. These and other lipophilic substances discovered in yeast are implicated in the regulation of growth, possibly through effects on the activation of the first step of protein synthesis -- the aminoacyl-tRNA synthetase reaction.

. . . . . Dr. S. Black

Protein Sequences and Structure. Cooperative binding systems involving aggregation, redistribution, subunit interactions, and ligand interactions are being formulated and studied in proteins related to hormone action.

Monte Carlo analyses are done to yield probabilities for uniqueness and homology of amino acid sequences. Our present studies in this area are concentrated on the relationship of uniqueness and the immune response.

. . . . . Dr. H. A. Saroff

Studies of the reaction of formaldehyde with amino acids and diamines have been continued. The equilibrium constants for ring formation from 2,4-diaminobutyric acid and from asparagine were measured.

An NMR method for determining the microscopic ionization constants in compounds with two ionizing groups was investigated. The precision of the method depends on the relatively large contribution of a nearby ionization to the chemical shift of a proton or carbon.

. . . . . Dr. R. B. Simpson

VII. HORMONES AND RECEPTORS

Insulin-Like Growth Factors: Biosynthesis and Action. We have continued our studies of the insulin-like growth factor/somatomedin, known as multiplication stimulating activity (MSA), the rat homologue of IGF-II, a polypeptide synthesized by a cloned line of rat liver cells (BRL-3A). During the past year, progress has been made in the following areas: (1) demonstration of a biosynthetic precursor of MSA (presumably pre-pro-MSA) in a cell-free translation system and of pro-MSA in intact metabolically-labeled BRL-3A cells; (2) demonstration by affinity crosslinking of two IGF receptor subtypes that differ in reactivity with insulin, IGF-I, and IGF-II, and in subunit structure; (3)

confirmation of the proposed IGF-II receptor structure by receptor purification (4) demonstration that insulin and MSA utilize insulin and IGF receptors respectively, to mediate the same biological responses in hepatocytes, chondrocytes, myocytes, fibroblasts, and embryonal carcinoma cells; (5) demonstration that the type I IGF receptor of a rat liver cell line has intrinsic tyrosine kinase activity and autophosphorylates the Mr 98K β-subunit of the receptor; (6) demonstration that type I IGF receptors, but not type II receptors, are selectively downregulated in a mouse muscle cell line; (7) demonstration that insulin acutely increases the number of type II IGF receptors on the surface of intact rat adipocytes by redistribution of cycling receptors from a large intracellular pool; (8) demonstration that insulin desensitizes rat hepatoma cells to IGF-stimulated IGF-receptor-mediated action at a step distal to hormone binding; (9) demonstration that IGF-II is the major somatomedin in fetal rat serum and that IGF-I levels increase shortly after birth; (10) demonstration that fetal/neonatal rat fibroblasts in culture synthesize IGF-II that is induced by ovine placental lactogen, whereas adult fibroblasts synthesize predominantly IGF-I induced by both growth hormone and placental lactogen; and (11) characterization of growth hormone-dependent binding proteins for somatomedins in human serum and fetal and postnatal rat serum.

. . . . . Drs. M. M. Rechler, M. A. De Vroede, D. E. Graham, N. Sasaki, and H. J. Whitfield, and J. A. Romanus, Y. W.-H. Yang (LBP, NIADDK); G. P. August, S. P. Nissley (MB, NCI); S. W. Cushman, I. A. Simpson, L. J. Wardzala (CMOS, NIADDK); G. Grunberger, R. W. Rees-Jones, Y. Zick (DB, NIADDK); C. R. Kahn, M. Kasuga (Joslin Diabetes Center, Boston); C. B. Bruni (University of Naples, Italy); J. M. Alvarez, T. D. Gelehrter, J. H. Heaton, N. L. Krett (University of Michigan, Ann Arbor)

Thyrotropin Receptors, Thyroid Regulation, and Pathophysiology. The structural components of the thyrotropin (TSH) receptor and their linkage to message transmitting signals continue to be defined. Reconstitution studies show that the specific ganglioside component of the receptor from thyroid cells can return thyroid-stimulating antibodies (TSAb) and TSH functions to normal in a thyroid tumor cell line with a defect in receptor-mediated adenylate cyclase stimulation. The specific ganglioside has been identified by autoradiography as a disialoganglioside only 0.1% of the total ganglioside pool. Monoclonal TSAbs from lymphocytes of patients which interact with gangliosides are not inhibited by monoclonal antibodies to the glycoprotein receptor components which inhibit TSH stimulation. Thus, the ganglioside component is a distinct entity of the TSH receptor with which TSH interacts after the glycoprotein receptor component, since TSAb stimulation exhibits competitive antagonism with respect to TSH. Clinical assays using a rat thyroid FRTL-5 cell line have been shown to measure growth and iodide uptake by TSAbs in 100% of patients with Graves' disease and to be predictive of therapeutic response. The growth of thyroid cells has been shown to depend on phospholipid and cAMP signals. Antibodies to the glycoprotein TSH receptor component initiate the phospholipid signal; TSAbs to the ganglioside initiate the cAMP signal. Only the former group is indomethacin-sensitive. Growth IgGs have been shown in multinodular goiter patients of select "genetic" types. The patent for assays has been granted and will be commercially developed under a license issued to the University of Maryland. TSH-Mediated growth stimulation has been linked to modulation of phospholipid turnover involving arachidonic acid metabolites. TSH-Stimulated iodide uptake has been linked to cAMP, protein synthesis, and regulation at an mRNA level. Iodide efflux is linked to leukotriene metabolism.

. . . . . Drs. S. M. Aloj, E. F. Grollman, C. Marcocci, D. Corda-Luini, C. M. Rotella, L. D. Kohn; Dr. W. A. Valente (University of Maryland Medical School, Baltimore); Dr. P. Laccetti (University of Naples, Italy); Dr. R. Toccafondi (University of Florence, Italy)

Tetanus Toxin Receptors and Mechanism of Action. Ganglioside-dependent axonal transport has been demonstrated; a ternary tetanus-ganglioside-cell glycoprotein complex has been isolated. Na$^+$-Dependent, Ca^{2+} flux modulation by tetanus toxin is amiloride-sensitive. Tetanus toxin modulation of lipid methylation and polyphosphoinositide turnover has been documented.

. . . . . Dr. L. D. Kohn; Drs. M. C. Hardegree, W. H. Habig (DBP, NCDB, FDA); Dr. E. Yavin (Weizmann Institute of Science, Rehovot, Israel)

Interferon Receptors and Action. Interferon- and TSH-mediated effects on thymidine uptake have been linked to an ADP ribosylation pathway. Interferon perturbations and TSH effects on thymidine uptake are cell density-dependent.

. . . . . Dr. L. D. Kohn

Graves' Disease. Additional human Graves' monoclonal antibodies have been obtained and characterized using a heterohybridoma approach. Additional human monoclonal Graves' stimulators have been characterized. Antibodies stimulating thyroidal growth and not adenylate cyclase have been defined and linked to phospholipid signals (see above). A select group of monoclonal antibodies to the TSH receptor glycoprotein component perturb collagen biosynthesis in fibroblasts. The assay measures exophthalmogenic IgGs. Nonthyroidal TSH receptor expression in connective tissue complications of Graves' disease are thus confirmed.

. . . . . . Dr. W. A. Valente (University of Maryland Medical School, Baltimore); Drs. C. M. Rotella, C. Marcocci, W. H. Hoffman, and L. D. Kohn

Thyroglobulin Biosynthesis and Degradation. Studies have defined a possible pathway of thyroglobulin biosynthesis involving the following steps. The 12 S ribosomal product after initial glycosylation by dolichol intermediates, but before remodeling of the B carbohydrate chain by addition of N-acetylglucosamine, galactose, and sialic acid, binds to thyroid membranes as a > 27 S moiety. Iodination of this material can stabilize the 27 S structure; alternatively, continued B carbohydrate remodeling leads to the formation of 19 S molecules. Both modified 27 S and 19 S forms then interact with different membrane binding sites which lead to vectoral transport to the follicular lumen and to iodination. Iodine residues in the 19 S and 27 S membrane-bound forms all exist as T_3/T_4 and in one 26 K peptide which can be released before the thyroglobulin is stored in the follicular lumen. This peptide is released as a membrane-bound component in Graves' and Hashimoto's thyroids. It is shown by monensin perturbation-pulse chase experiments that this represents a short-loop release process which is exaggerated in these diseases and can account for hyperthyroidism and Hashitoxicosis. The 26 K T_3/T_4-rich peptide has been characterized and shown to exhibit unusual fluorescence and spectral properties linked to a unique class of iodotyrosine-iodotyrosine interactions which ha been predicted to be important in T_3/T_4 formation. The phosphate thyroglobuli receptor has been purified and phosphate residues on thyroglobulin characterized as tyrosine PO_4 (10-20%) and sugar PO_4 (80-90%). The sugar PO_4 structure is sugar 6 PO_4 1 GlcNAc.

. . . . . Drs. M. L. De Luca, P. Santisteban, L. D. Kohn; Dr. S. Shifrin (DCBD, NCI); Dr. E. Consiglio (University of Naples, Italy)

Electrochemical Ion Gradients as a Mechanism of Cellular Message Transmission. The work relates to our understanding of the biochemical events associated with the normal function of the thyroid and to pathological conditions of the thyroid, such as in Graves' disease. The work began with a study of the effect of thyrotropin (TSH) on iodide transport mechanisms. The work has evolved in several directions: (1) the role of phospholipid metabolism in calcium mobilization, iodide efflux, and ligand-stimulated growth; (2) a detailed study of protein synthesis reflecting mRNAs induced by TSH and cyclic AMP; and (3) effects on iodide fluxes that can be related to, and reflect receptor activation by, a variety of ligands. The work continues to support the hypothesis that alterations in ion fluxes are important early events, as well as primary actions of TSH and pharmacological agents.

. . . . . Drs. E. F. Grollman, C. Marcocci, D. Tombaccini, and F. V. Alvarez

VIII. BLOOD COAGULATION

Addition of certain plasma proteins to the thrombin conversion of fibrinogen fibrin results in substantial decreases in clotting times. This does not appe to be due to enhanced thrombin action or coaggregation of the added protein with fibrin/fibrinogen.

. . . . . Dr. J. A. Gladner

IX. CULTURE OF MARROW CELLS AND OF MYCOBACTERIUM

Efforts were made to improve the growth of mouse megakaryocytes in cultures. Now good granular megakaryocytes are regularly observed on day 0. A few cells showing beautiful demarcation membrane formation are observed on day 2 to 3. Marked increases in the number of cells, mostly the megakaryoblasts, appear on day 3, reaching a peak on day 5 to 6. Apparently 500 megakaryocytes per 2×10^5 nucleated marrow cells are obtained at this stage. Well developed promegakaryocytes are routinely observed on day 4 to 5, and quite a few granular megakaryocytes are also observed at this stage. Unfortunately, the latest mature form, cells showing demarcation membrane systems, is observed only infrequently. Studies to improve cell maturation are in progress.

. . . . . Dr. Y. T. Chang

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 23,140-26 LBP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemistry of Sulfur-Containing Compounds

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Simon Black, Ph.D.	Biochemist and Assistant Chief, LBP	LBP NIADDK
Others:	Chien-Hua Niu, Ph.D.	Senior Staff Fellow	LBP NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Pharmacology

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 2.8	PROFESSIONAL: 1.8	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Evolution. Hydrogen bonding between the purines, adenine and guanine, and the tripeptide, triglycine, has been demonstrated. Binding in aqueous solution between the peptide Lys-Ser-Gly-Lys and a newly synthesized 10-member oligonucleotide of cytosine has been shown by measuring the circular dichroism spectrum of (dC)10.

Protein Synthesis. The activation of a valyl-tRNA synthetase discovered in this laboratory has been found to be powerfully inhibited by unsaturated fatty acids. These and other lipophilic substances discovered in yeast are implicated in the regulation of growth, possibly through effects on the activation of the first step of protein synthesis -- the aminoacyl-tRNA synthetase reaction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 23,230-34 LBP

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chemotherapy of Mouse Leprosy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Yao Teh Chang, M.D. Research Pharmacologist LBP NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Efforts were made to improve the growth of mouse megakaryocytes in cultures. Now good granular megakaryocytes are regularly observed on day 0. A few cells showing beautiful demarcation membrane formation are observed on day 2 to 3. Marked increases in the number of cells, mostly the megakaryoblasts, appear on day 3, reaching a peak on day 5 to 6. Apparently 500 megakaryocytes per 2 x 10 to the 5th power nucleated marrow cells are obtained at this stage. Well developed promegakaryocytes are routinely observed on day 4 to 5, and quite a few granular megakaryocytes are also observed at this stage. Unfortunately, the latest mature form, cells showing demarcation membrane systems, is observed only infrequently. Studies to improve cell maturation are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 23,330-06 LBP

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Aldoheptose Biosynthesis and Its Regulation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	William G. Coleman, Jr., Ph.D.	Research Microbiologist	LBP NIADDK
Others:	Joyce C. Pegues, Ph.D.	Staff Fellow	LBP NIADDK

COOPERATING UNITS *(if any)*

Belinda P. Seto, Ph.D., Research Chemist, DBBP, NCDB, FDA

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Laboratory of Biochemical Pharmacology

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
2.1	1.6	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previously, a novobiocin-hypersensitive mutant of Escherichia coli K-12 carrying a cysE-pyrE linked mutation, designated rfaD, which specifically affects the synthesis of the aldoheptose, L-glycero-D-mannoheptose, has been isolated and genetically characterized. The following facts have been established: (1) The truncated lipopolysaccharide (LPS) of the rfaD mutant contains D-glycero-D-mannoheptose rather than the normal L-glycero-D-mannoheptose. (2) The rfaD gene product, ADP-L-glycero-D-mannoheptose-6-epimerase, is required for the conversion of ADP-D-glycero-D-mannoheptose to ADP-L-glycero-D-mannoheptose. (3) The nucleotide ADP-D-glycero-D-mannoheptose accumulates in the rfaD strains. These results provide support for the following biosynthetic reactions: (a) the conversion of D-glycero-D-mannoheptose-1-P and ATP to ADP-D-glycero-D-mannoheptose and pyrophosphate and (b) ADP-D-glycero-D-mannoheptose is converted to ADP-L-glycero-D-mannoheptose. These two reactions are catalyzed by the enzymes ADP-D-glycero-D-mannoheptose-synthetase and ADP-L-glycero-D-mannoheptose-6-epimerase, respectively.

The rfaD gene has been cloned into pBR322, and the rfaD gene product has been partially purified from plasmid amplified strains.

Recently, we have isolated a new mutant carrying a cysE-pyrE linked mutation, designated rfa-2, which results in an increased permeability to hydrophobic agents and a heptose-less LPS structure. Further, we have demonstrated that the rfa-2 mutation is genetically distinct from the rfaD locus and the rfa-2 phenotype is not abolished by plasmid carrying the wild type rfaD allele. The rfaD gene product, ADP-L-glycero-D-mannoheptose-6-epimerase, is detectable in crude extracts of rfa-2 mutant strains. An automated sugar analyzer has been developed in our laborator which allows us to follow directly the conversion of the sugar intermediate involved in the biosynthesis of L-glycero-D-mannoheptose.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 23,580-21 LBP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Gene Expression in the Rat and Other Organisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Anthony V. Furano, M.D.	Medical Officer (Research) and Chief, Section on Genomic Structure and Function, LBP	LBP NIADDK
Others:	Ettore D'Ambrosio, Ph.D.	Visiting Fellow	LBP NIADDK
	Malathi S. Lakshmikumaran, Ph.D.	Visiting Associate	LBP NIADDK
	Anna Lo Cuoco	Guest Researcher	LBP NIADDK
	Anne Salemme, B.A.	Guest Researcher	LBP NIADDK

COOPERATING UNITS *(if any)*

Dr. Philip Tsichlis, Fox Chase Cancer Center, Philadelphia, Pennsylvania
Dr. Robert H. Bassin, Laboratory of Tumor Immunology and Biology, NCI, NIH

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Genomic Structure and Function

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.9	2.8	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

All metazoans examined to date contain families of repetitive DNA sequences, and suggestions for their function include coordination of the expression of tissue-specific genes, promotion of chromosomal rearrangements such as duplications, inversions, and deletions, a role in speciation, or even no function except self-propagation as "selfish" DNA. We have developed an experimental approach to rapidly characterize and identify rat repeated DNA sequences of potential interest, and in our earlier studies we identified a number of highly repeated, transcribed DNA sequences that appear to be organized as permuted clusters. In examining the genomic regions that contain these sequences we found that one class of them is located in long, 3-7 kb structures that are themselves repeated about 50,000 times in the rat genome. These structures are the counterparts in rats of long interspersed repeated elements (referred to as LINES) that have been identified in mice and primates. We have determined their overall structure and found that, although they are quite polymorphic, they share common architectural features as revealed by the spacing of certain restriction enzymes sites. In addition we have shown that rat LINES appear to be mobile in the rat genome since they produce heterozygosity among individual rats of different laboratory strains as well as of wild rats. Three such loci are the murine leukemia virus integration site 2, the immunoglobulin heavy chain locus, and the rat insulin I locus. The latter case is particularly intriguing because amplification of the rat insulin I locus and flanking DNA accompanies the presence of the rat LINE. We also found that rat repeated sequences can be classified as either "G:C-rich" or "A:T-rich". The latter are the ones that comprise rat LINES, and genomic regions that contain LINES contain few, if any, of the G:C-rich sequences. On the other hand, the regions of the genome that contain multiple copies of the G:C-rich sequences appear to lack LINES. In an attempt to confirm the impression that G:C-rich and A:T-rich repeated sequences are segregated in the genome, we are using in situ hybridization to map the G:C-rich and A:T-rich repeated sequences at the chromosomal level.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 23,630-19 LBP

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biology of Complex Carbohydrates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Victor Ginsburg, Ph.D.	Research Chemist and Chief, Section on Biochemistry, LBP	LBP NIADDK
Others:	Pam L. Fredman, Ph.D.	Visiting Associate	LBP NIADDK
	John L. Magnani, Ph.D.	Senior Staff Fellow	LBP NIADDK
	David D. Roberts, Ph.D.	Staff Fellow	LBP NIADDK
	Patrice F. Spitalnik, M.D.	Guest Researcher	LBP NIADDK
	Steven L. Spitalnik, M.D.	Medical Staff Fellow	LBP NIADDK

COOPERATING UNITS *(if any)*

None

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SECTION
Section on Biochemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
6.5	4.5	2.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cell surface carbohydrates change during development as they are probably involved in differentiation. These developmentally-regulated changes allow some antibodies directed against carbohydrates to discriminate among tissues, both normal and malignant. To obtain monoclonal antibodies specific for various cell types, mice and rats have been immunized with human tissues in many laboratories. Some of the antibodies derived from spleen cells of the immunized animals have an apparent specificity for certain cancers. Out of 460 of these antibodies sent to us from several laboratories in the past 4 years, 121 are directed against carbohydrate sequences in glycoproteins and glycolipids as determined by solid-phase radioimmunoassay and by immunostaining of thin layer chromatograms of glycolipids. Of the 121 antibodies, 23 are directed against human blood group antigens including 7 against the H type 1 or H type 2 antigens, 4 against the Le-b antigen, 1 against the Le-a antigen, 8 against the A antigen, and 3 against the B antigen. Fifty-six antibodies are directed against a sugar sequence found in the human milk oligosaccharide lacto-N-fucopentaose III, 1 against a sugar sequence found in the human milk oligosaccharide lacto-N-neotetraose, 2 against a sialylated Le-a antigen, and 40 against unidentified carbohydrate sequences in glycolipids and/or glycoproteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 23,730-24 LBP

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Factors Involved in Blood Coagulation and Related Fields

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Jules A. Gladner, Ph.D. Guest Researcher LBP NIADDK

COOPERATING UNITS *(if any)*

Ralph J. Nossal, Ph.D., Biophysicist, PSL, DCRT, and Allen P. Minton, Research Chemist, LBP, NIADDK

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SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.0	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Addition of certain plasma proteins to the thrombin conversion of fibrinogen to fibrin results in substantial decreases in clotting times. This does not appear to be due to enhanced thrombin action or coaggregation of the added protein with fibrin/fibrinogen.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 23,830-05 LBP

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Electrochemical Ion Gradients as a Mechanism of Cellular Message Transmission

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Evelyn F. Grollman, M.D.	Medical Officer (Research)	LBP NIADDK
Others:	Francisco V. Alvarez, Ph.D.	Guest Researcher	LBP NIADDK
	Claudio Marcocci, M.D.	Visiting Associate	LBP NIADDK
	Donatella Tombaccini, Ph.D.	Guest Researcher	LBP NIADDK

COOPERATING UNITS *(if any)*
R. M. Friedman, LEP, NIADDK; W. H. Habig, BTB, DEP, BB, FDA; M. C. Hardegree, BTB, DEP, BB, FDA; N. J. Philp, University of Pennsylvania, Philadelphia, Pennsylvania

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Laboratory of Biochemical Pharmacology

SECTION
Section on Biochemistry of Cell Regulation

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.4	3.2	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The work relates to our understanding of the biochemical events associated with the normal function of the thyroid and to pathological conditions of the thyroid, such as in Graves' disease. The work began with a study of the effect of thyrotropin (TSH) on iodide transport mechanisms. The work has evolved in several directions: (1) the role of phospholipid metabolism in calcium mobilization, iodide efflux, and ligand-stimulated growth; (2) a detailed study of protein synthesis reflecting mRNAs induced by TSH and cyclic AMP; and (3) effects on iodide fluxes that can be related to, and reflect receptor activation by, a variety of ligands. The work continues to support the hypothesis that alterations in ion fluxes are important early events, as well as primary actions of TSH and pharmacological agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 23,960-18 LBP

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Cell Regulation by the Action of Pharmacodynamic Agents on the Cell Membrane

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Leonard D. Kohn, M.D.	Medical Director, USPHS, and Chief, Section on Biochemistry of Cell Regulation	LBP NIADDK
Others:	Salvatore M. Aloj, M.D.	Visiting Scientist	LBP NIADDK
	Michele L. De Luca, M.D.	Visiting Fellow	LBP NIADDK
	William H. Hoffman, M.D.	Expert	LBP NIADDK
	Daniela Corda Luini, Ph.D.	Visiting Fellow	LBP NIADDK
	Carl M. Rotella, M.D.	Visiting Associate	LBP NIADDK
	Pilar Santisteban, M.D.	Guest Researcher	LBP NIADDK

COOPERATING UNITS *(if any)* S. Shifrin, DCBD,NCI; E. F. Grollman, C. Marcocci, LBP,NIADDK; H. J. C. Yeh, LC,NIADDK; E. Yavin, Weizmann Inst., Rehovot, Israel; W. A. Valente, Univ. of Md., Baltimore; W. H. Habig, M. C. Hardegree, BB,FDA; E. Consiglio and P. Laccetti, Univ. of Naples, Italy; R. Toccafondi, Univ. of Florence, Italy

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Laboratory of Biochemical Pharmacology

SECTION
Section on Biochemistry of Cell Regulation

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.3	6.2	1.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The structural and functional relationship in the mechanism by which glycoprotein hormones [thyrotropin (TSH), luteinizing hormone (LH), human chorionic gonadotropin (hCG), and follicle-stimulating hormone (FSH)], certain bacterial toxins (cholera and tetanus, for example), and the antiviral protective agent, interferon, interact with and transmit their message through the cell membrane, is being further defined. Studies using monoclonal antibodies have continued which explore the importance of these relationships to the expression of thyroid hyperfunction in Graves' disease; to organ-specific autoimmunity in general and the autoimmunity of Graves' disease in particular; to fluid losses in intestinal diarrhetic states; to thyroid storm and the sympathetic overactivity syndrome of tetanus; to the ability of hormones to modulate the oncogenic state; and to the mechanism by which toxins subvert normal mechanisms to impose their pathological effects. Studies have been continued which evaluate the role of membranes in thyroglobulin biosynthesis and thyroglobulin biodegradation to T3 and T4 and the role of carbohydrate moieties in thyroglobulin structure and post-translational processing. Studies also continue to explore lipid regulation of receptor expression with special emphasis on neuronal and thyroid cell growth and development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 24,120-13 LBP

PERIOD COVERED
October 1, 1983 through May 7, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanism of Hypertension in Rats

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Sanford M. Rosenthal, M.D. Scientist Emeritus LBP NIADDK

COOPERATING UNITS *(if any)*

None

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Laboratory of Biochemical Pharmacology

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was terminated on May 7, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 24,140-18 LBP

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Tryptophan Synthase: Structure, Function, and Subunit Interactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Edith Wilson Miles, Ph.D.	Research Chemist	LBP NIADDK
Others:	Syed A. Ahmed, Ph.D.	Visiting Fellow	LBP NIADDK
	Hyone-Myong Eun, Ph.D.	Visiting Fellow	LBP NIADDK

COOPERATING UNITS *(if any)* Dr. K. Yutani, Inst. for Protein Res. Osaka Univ. Japan; Dr. T. Fairwell, MD, NHLBI; Dr. D.R. Davies, LMB, NIADDK; Drs. R.S. Phillips & L.A. Cohen, LC, NIADDK; Drs. N. Esaki; H. Tanaka & K. Soda, Inst. for Chem. Res. Kyoto Univ. Japan; Dr. R. Bauerle, Dept. of Biol., Univ. Virginia, Charlottesville, VA.

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Section on Pharmacology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.3	3.0	0.3

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The tryptophan synthase α2β2 complex is a good model for other multienzyme complexes, including hormone receptors and complexes which synthesize DNA or fatty acids. We are investigating the structure of the α2β2 complex and of the component α and β2 subunits from 3 different bacterial sources (Escherichia coli, Salmonella typhimurium, and an interspecies hybrid) using studies of solubility, folding properties, domain structure, and susceptibility to limited proteolysis. These comparative studies are aimed at understanding the effects of the divergent evolution of E. coli and S. typhimurium upon the structure and function of enzymes. Most recently we have initiated X-ray crystallographic studies of the α2β2 complex from S. typhimurium which should lead to a much greater understanding of the interaction between the α and β2 subunits and of their separate active sites.

We are also studying the function of tryptophan synthase using chemical modification and investigations with a variety of substrates and substrate analogs. An arginyl residue in the α subunit is modified by phenylglyoxal and protected from modification by the substrate, indole-3-glycerol phosphate. We have discovered that phenylgyoxal is not specific for arginine, as has been generally thought, but also reacts with some sulfhydryl residues in proteins. Our finding that two tryptophan analogs, oxindolyl-L-alanine and 2,3-dihydro-L-tryptophan, are potent competitive inhibitors of both tryptophan synthase and tryptophanase is evidence that the indolenine tautomer of L-tryptophan, which resembles the inhibitors in its geometry at C-3 of the heterocyclic ring, is an intermediate in the reactions catalyzed by both enzymes. 6-Azido-L-tryptophan is an effective photoaffinity inhibitor. Studies of the substrate and reaction specificity of tryptophan show that the enzyme can synthesize a number of S-substituted L-cysteine, S-substituted β-methylcysteine, and Se-substituted L-selenocysteine derivatives by β-replacement reactions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 24,150-13 LBP

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Noncovalent Intermolecular Interactions in Biochemistry

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Allen P. Minton, Ph.D.	Reseach Chemist	LBP NIADDK
Others:	Arun K. Attri, Ph.D.	Visiting Fellow	LBP NIADDK
	Jacob Wilf, Ph.D.	Visiting Associate	LBP NIADDK

COOPERATING UNITS *(if any)*

Jules A. Gladner, Ph.D., Guest Researcher, LBP, NIADDK

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Laboratory of Biochemical Pharmacology

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	2.6	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Data taken from the literature on receptor-mediated stimulation of adenylate cyclase have been reviewed and analyzed to determine whether and to what extent these data are compatible with several previously proposed mechanistic molecular models and extensions thereof.

The kinetics of appearance of fibrinopeptides following the addition of thrombin or other specific enzymes to fibrinogen are being measured via amino acid analysis of fibrinopeptide hydrolyzates.

The kinetics of appearance of small soluble oligomers of fibrinogen/fibrin at early times in the fibrin gelation reaction are being measured via a novel application of high performance gel exclusion chromatography.

The dependence of fibrin gelation time upon initial concentrations of fibrinogen and thrombin, upon the delay time preceding addition of a thrombin inhibitor, and upon the concentration of unrelated globular proteins present in the reaction mixture, is being measured using a novel viscometric technique.

An automated method is being developed for measuring the concentration gradient of individual macromolecular species in a centrifuged solution containing several macromolecular solutes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 24,260-18 LBP

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Enzymatic Mechanisms of DNA Replication: The Bacteriophage T4 System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Nancy G. Nossal, Ph.D.	Research Chemist and Chief, Section on Nucleic Acid Biochemistry	LBP NIADDK
Others:	Deborah M. Hinton, Ph.D.	Senior Staff Fellow	LBP NIADDK
	Ross W. Richardson, Ph.D.	Staff Fellow	LBP NIADDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Nucleic Acid Biochemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.7	2.2	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are studying the *Escherichia coli* bacteriophage T4 as a model system for duplex DNA replication. Efficient DNA synthesis *in vitro* can be achieved with a mixture of seven purified proteins encoded by T4 phage, which include T4 DNA polymerase (gene 43 product), the gene 32 DNA helix destabilizing protein, the gene 44/62 and gene 45 polymerase accessory proteins, and the gene 41 and gene 61 priming proteins. We are continuing our studies to determine in detail the function of each of these proteins in DNA synthesis, and the processes used to coordinate their activities.

We have constructed pBR322-based plasmids with T4 gene 41 downstream of the PL promoter that produce 100-fold more gene 41 priming protein than is present in an infected cell. Plasmids with T4 DNA from 15.0 to 18.7 map units express gene 61 primase activity.

Plasmids containing T4 DNA upstream of gene 41 produce a 46 kd protein that binds tightly to double-stranded DNA. A protein of the same size is made by *in vitro* transcription-translation of DNA from these plasmids or T4 DNA restriction fragments from the same region. The 46 kd gene is located about 500 bp downstream of two strong promoters detected *in vitro*. The 46 kd protein appears to be encoded or in some way controlled by the T4 *uvsX* gene. T4 *fdsA* mutant in the *uvsX* gene has been shown by Dewey and Frankel to suppress T4 mutants in the gene 49 nuclease. We find that this suppression does not occur in cells containing plasmids that produce the 46 kd protein. Moreover, the 46 kd dsDNA binding protein in extracts of cells infected with wild type T4 is not found after infection with the *fdsA* or *am* 11 mutants in the *uvsX* gene.

We are continuing our efforts to develop an *in vitro* system to study the initiation of T4 DNA replication. We have found conditions under which fractions from T4 infected cells show a preference for synthesis with T4 DNA compared to other linear dsDNA. This DNA synthesis is dependent on the T4 gene 41 and 45 replication proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 24,360-10 LBP

PERIOD COVERED
October 1, 1983 through January 6, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Action of Thrombin on Fibrinogen and Study of Related Blood Clotting Mechanisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Albert J. Osbahr, Ph.D.	Research Biochemist	LBP NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Genomic Structure and Function

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated due to the retirement of Dr. Albert J. Osbahr on January 6, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 24,390-11 LBP

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Hormones and Receptors: Insulin and Insulin-Like Growth Factors (Somatomedins)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Matthew M. Rechler, M.D.	Medical Director, USPHS	LBP NIADDK
Others:	Monique A. De Vroede, M.D.	Visiting Associate	LBP NIADDK
	Dale E. Graham, Ph.D.	Special Expert	LBP NIADDK
	Joyce A. Romanus, B.A.	Biologist	LBP NIADDK
	Norio Sasaki, M.D.	Visiting Associate	LBP NIADDK
	Harvey J. Whitfield, M.D.	Special Expert	LBP NIADDK
	Yvonne W.-H. Yang, B.S.	Guest Researcher	LBP NIADDK

COOPERATING UNITS *(if any)* G.P. August, S.P. Nissley, MB,NCI; S.W. Cushman, I.A. Simpson, L.J. Wardzala, CMOS,NIADDK; G. Grunberger, R.W. Rees-Jones, Y. Zick, DB,NIADDK; C.R. Kahn, M. Kasuga, Joslin Diabetes Center, Boston; C.B. Bruni, Univ. of Naples, Italy; J.M. Alvarez, T.D. Gelehrter, J.H. Heaton, N.L. Krett, Univ. of Michigan

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Biochemistry of Cell Regulation

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.5	6.0	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have continued our studies of the insulin-like growth factor/somatomedin, known as multiplication stimulating activity (MSA), the rat homologue of IGF-II, a polypeptide synthesized by a cloned line of rat liver cells (BRL-3A). During the past year, progress has been made in the following areas: (1) demonstration of a biosynthetic precursor of MSA (presumably pre-pro-MSA) in a cell-free translation system and of pro-MSA in intact metabolically-labeled BRL-3A cells; (2) demonstration by affinity crosslinking of two IGF receptor subtypes that differ in reactivity with insulin, IGF-I, and IGF-II, and in subunit structure; (3) confirmation of the proposed IGF-II receptor structure by receptor purification; (4) demonstration that insulin and MSA utilize insulin and IGF receptors, respectively, to mediate the same biological responses in hepatocytes, chondrocytes, myocytes, fibroblasts, and embryonal carcinoma cells; (5) demonstration that the type I IGF receptor of a rat liver cell line has intrinsic tyrosine kinase activity and autophosphorylates the Mr 98K β-subunit of the receptor; (6) demonstration that type I IGF receptors, but not type II receptors, are selectively downregulated in a mouse muscle cell line; (7) demonstration that insulin acutely increases the number of type II IGF receptors on the surface of intact rat adipocytes by redistribution of cycling receptors from a large intracellular pool; (8) demonstration that insulin desensitizes rat hepatoma cells to IGF-stimulated IGF-receptor-mediated action at a step distal to hormone binding; (9) demonstration that IGF-II is the major somatomedin in fetal rat serum and that IGF-I levels increase shortly after birth; (10) demonstration that fetal/neonatal rat fibroblasts in culture synthesize IGF-II that is induced by ovine placental lactogen, whereas adult fibroblasts synthesize predominantly IGF-I induced by both growth hormone and placental lactogen; and (11) characterization of growth hormone-dependent binding proteins for somatomedins in human serum and fetal and postnatal rat serum.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 24,590-13 LBP

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
Structure and Interactions of Biologically Important Macromolecules

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

PI:	Harry A. Saroff, Ph.D.	Scientist Emeritus and Special Expert	LBP NIADDK

COOPERATING UNITS (*if any*)
Clinical Endocrinology Branch, NIADDK, NIH; Laboratory of Immunogenetics, NIAID, NIH; National Center for Drugs and Biologics

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.1	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Cooperative binding systems involving aggregation, redistribution, subunit interactions, and ligand interactions are being formulated and studied in proteins related to hormone action.

Monte Carlo analyses are done to yield probabilities for uniqueness and homology of amino acid sequences. Our present studies in this area are concentrated on the relationship of uniqueness and the immune response.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 24,640-13 LBP

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
(I) Polarography of Carcinogens and (II) Reactions of Formaldehyde

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Richard B. Simpson, Ph.D.	Guest Researcher	LBP NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	1.0	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies of the reaction of formaldehyde with amino acids and diamines have been continued. The equilibrium constants for ring formation from 2,4-diaminobutyric acid and from asparagine were measured.

An NMR method for determining the microscopic ionization constants in compounds with two ionizing groups was investigated. The precision of the method depends on the relatively large contribution of a nearby ionization to the chemical shift of a proton or carbon.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 24,709-03 LBP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Purification of Ornithine Decarboxylase and Regulation of S. cerevisiae

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Celia White Tabor, M.D. Medical Director, USPHS LBP NIADDK

COOPERATING UNITS *(if any)*

Drs. H. Tabor and R. B. Wickner, LBP, NIADDK

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

Section on Pharmacology

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 2.5	PROFESSIONAL: 1.0	OTHER: 1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have continued our studies on the biosynthesis, regulation, and function of polyamines in Saccharomyces cerevisiae. Our major effort during the past year has been on studying ornithine decarboxylase, the first step in the biosynthetic pathway for polyamines in yeast. We are studying the mechanisms of control in this system as a model for the eukaryotic enzyme, which has been of special interest because it increases very rapidly under conditions of rapid growth and differentiation.

We have been studying five problems related to this enzyme: (1) purification of the enzyme; (2) regulatory control of enzymatic activity; (3) genetics of the enzyme; (4) development of methods to facilitate isolation of clones carrying the gene; and (5) physiologic effects of polyamine deprivation.

(1) Procedures have been developed for the purification of a stable form of an 86,000 Mr protein, which is secondarily converted by proteolytic enzymes to a 68,000 Mr and a 35,000 Mr form. The enzyme has been shown to be a glycoprotein. (2) The enzyme loses activity after growth in the presence of polyamines; no obvious protein degradation is involved and the regulation is by a putative post-translational modification. (3) We have shown that all of the spe10 mutants, which lack ornithine decarboxylase activity, are located in the same genetic locus. It is still uncertain if this defect is in a regulatory or a structural gene. (4) A screening technique has been developed to select for strains that might have a recombinant plasmid carrying the gene for ornithine decarboxylase (or other genes in the polyamine pathway). Banks of recombinant plasmids have been constructed. (5) We have also been using the various mutants that we previously described to study the physiologic effect of polyamine deprivation. We have found that amine-deficient cells arrest in a budded stage, similar to cell division cycle mutants.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 24,710-34 LBP

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Estimation, Metabolism, and Function of Amines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Herbert Tabor, M.D. Supervisory Medical Officer (Research); Chief, Laboratory of Biochemical Pharmacology; Chief, Section on Pharmacology, LBP LBP NIADDK

Others: Sushil K. Taneja, Ph.D. Visiting Associate LBP NIADDK

COOPERATING UNITS *(if any)*

Dr. C. W. Tabor, LBP, NIADDK

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Pharmacology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.5	2.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our overall studies have been concerned with the biosynthesis, metabolism, and function of the polyamines, particularly putrescine and spermidine. In this part of the work we have concentrated on the biosynthesis and function of polyamines in Escherichia coli. We have continued our studies on the specific genes and enzymatic steps involved in the biosynthesis of these amines. Mutants in these genes have been selected, and recombinant DNA plasmids have been constructed. Strains containing these plasmids have been used for the overproduction of the respective enzymes.

During the past year we have been particularly interested in the structure and mechanism of one of the enzymes involved in spermidine biosynthesis, namely, adenosylmethionine decarboxylase, which is coded for by the speD gene. We had previously shown that this enzyme contains covalently-linked pyruvate, and this moiety is required for activity; we are now studying the mechanism of formation of this unusual protein component. As part of this study the speD gene and the gene product (adenosylmethionine decarboxylase) are being sequenced, and better recombinant plasmids have been constructed for the overproduction of the enzyme. Antibodies have been prepared for the detection of the enzyme and any proenzyme in minicell preparations.

We have also been continuing our studies on the physiological role of these amines, using our mutants that lack all of the biosynthetic pathways. We have shown that polyamines affect the conformation or stability of the protein-synthesizing ribosomal complex *in vivo*. Studies have continued on the effects of polyamines on the growth of various bacteriophages and on their role in permitting the readthrough of amber mutants.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 24,940-11 LBP

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Killer Double-Stranded RNA Plasmids of Saccharomyces cerevisiae

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Reed B. Wickner, M.D.	Medical Director, USPHS, and Chief, Section on Genetics of Simple Eukaryotes	LBP NIADDK
Others:	M. Rosa Esteban Canibano, Ph.D.	Visiting Fellow	LBP NIADDK
	Tsutoma Fujimura, Ph.D.	Visiting Associate	LBP NIADDK
	Steve S. Sommer, M.D., Ph.D.	Medical Staff Fellow	LBP NIADDK

COOPERATING UNITS *(if any)*

Faculte des Sciences Agronomiques de l'Etat, B5800, Gembloux, Belgium

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION
Section on Genetics of Simple Eukaryotes

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.0	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Yeast carries up to five different dsRNAs, called L-A, L-(BC), T, W, and M. Killer strains of yeast secrete a protein toxin to which they are themselves immune. Toxin and immunity are determined by the M dsRNAs: M1 (1.5 kb) for the K1 system and M2 (1.0 kb) for the K2 system. The replication of M1 or M2 depends on L-A (4.5 kb), which encodes the major protein of the virus-like particles in which L-A and M dsRNAs are found. Thirty chromosomal genes (MAK) are necessary for M1 or M2 replication. Two genes (MKT) are specifically needed for M2 and only if L-A-HN is present. We have shown that M dsRNA represses L-A replication but does not affect L-(BC), while the six chromosomal SKI+ gene products repress L-A, L-(BC), and M dsRNA replication, as shown by 3- to 10-fold lowering of their copy numbers. The effect of M on L-A is epistatic to that of SKI products. We have isolated a ski- strain that can maintain M1 in spite of its lacking L-A completely. MAK1 has been shown by others to be the gene for yeast topoisomerase I. Our work showed this gene's involvement in M dsRNA replication (but not L-A, L-B, T, or W). We recently found that all three mutant alleles of MAK1 confer cold sensitivity for cell growth (8°C), although growth is normal from 20°C to 37°C. The gene for yeast topoisomerase II was located by others near MET4 on chromosome XIV. We recently mapped the MKT1 gene on chromosome XIV, also near MET4, and are investigating the possible identity of these two genes. They are at least very close to each other, as no recombination was found in a total of 41 meiotic tetrads. One allele of MKT1, when combined with a particular cytoplasmic element, results in cold sensitivity for cell growth (8°C). Preliminary evidence suggests that the cytoplasmic element is L-(BC). We previously showed that ski- mutants carrying M dsRNA are cold-sensitive for growth because their M copy number is elevated. Using the cold sensitivity of ski- strains carrying M dsRNA, we have cloned segments of yeast DNA that suppress this effect. These should include the SKI+ genes themselves and genes, overproduction of whose products suppress this phenotype. We have discovered a chromosomal gene affecting the copy number of both T and W dsRNAs.

ANNUAL REPORT OF THE LABORATORY OF CHEMICAL BIOLOGY

National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases

The Laboratory of Chemical Biology conducts research on fundamental problems of cell biology relating the chemistry of macromolecules to cell function; on the structure, function and dynamics of proteins; and on molecular biology and genetics, especially as related to genetic diseases. During the last three years an increasing fraction of the Laboratory's efforts have dealt with new programs in cellular, molecular and genetic biology, as well as new approaches to the study of protein conformational dynamics. These projects include biophysical, biochemical, and molecular genetic approaches to biological questions. Lastly, the program on sickle cell anemia is now involved in several clinical evaluation and treatment protocols, as well as basic molecular and cellular studies.

Among the major new areas of exploration in the Laboratory of Chemical Biology are the use of an erythroleukemic cell line as a model for studying developmental "switching" of endogenous globin genes, the expression of foreign genes transfected into these cells, and the structure of chromatin of transcriptionally active genes; and the isolation, characterization and use of yeast cytochrome c synthetase in order to synthesize an ancestral cytochrome c. NMR techniques are now being used to study the dynamics of these proteins and a new project on the specificity of antibody binding is being initiated. The program on the molecular pathogenesis of sickle cell anemia is involved in analyses of the origin and significance of erythrocyte heterogeneity; the project on the development of non-covalent inhibitors of sickle hemoglobin gelation has progressed to an analysis, by difference Fourier X-ray crystallography, of the binding of several gelation inhibitors to hemoglobin crystals; and the entire sickle cell program program is concentrating on new methods of evaluating disease severity and response to therapy in patients, including a clinical study of the use of 5-azacytidine and hydroxyurea to increase fetal hemoglobin levels.

Several major personnel changes have occurred during this year in the Laboratory of Chemical Biology. Dr. Adrian Parsegian has been detailed to the Laboratory by the Division of Computer Research and Technology to establish a program of experimental and theoretical research on the action of molecular forces governing macromolecular assembly, including molecular modeling using computer graphics. Personnel and other resources for this program will be shared by NIADDK and DCRT. Dr. Edward Steers has functioned as acting Deputy Intramural Director and his research program on platelets has been transferred to the Clinical Hematology Branch of this Institute. At the beginning of the year Dr. Jurrien Dean transferred his research program on the zona pellucida to the Laboratory of Cellular and Developmental Biology of NIADDK. At the end of the year Dr. Irwin Chaiken was transferred, with his research program on neurophysins, to a new group under the direction of Dr. Bruce Weintraub of this Institute.

Section on Macromolecular Biology

The efforts of this Section are concentrated in two major areas. One group is interested in the pathophysiology of sickle cell anemia and related hemoglobinopathies at the molecular, cellular and clinical levels. A second research group is working on human erythroid cell biology and molecular genetics, using the K562 erythroleukemic cell line as a model system.

The work on hemoglobin has centered on experimental and theoretical approaches to understanding the intracellular polymerization of sickle hemoglobin in sickle cell anemia and other sickle-syndromes. New methods of nuclear magnetic resonance spectroscopy, cell fractionation, and cell filterability measurements have been developed to study intracellular polymerization and new theoretical analyses involving protein and water non-ideality have been developed to explain the results and to make predictions about in vivo behavior. Several pilot clinical studies have been initiated, which are designed to predict the severity of sickle cell anemia in individuals and the response to therapy. These studies involve use of minimally invasive modalities, such as exercise tolerance, quantitative ophthalmological examinations, positron emission tomography, nuclear magnetic resonance imaging and laser-Doppler velocimetry to evaluate objectively blood flow in affected individuals. The work on gelation inhibitors has continued through the synthesis and assay of new sets of compounds which prevent the intermolecular assembly process, as well as through a collaborative study with the Medical Research Council Laboratory of Molecular Biology in Cambridge, England to determine the site of binding, and thus the mode of action at the atomic level, of several of the peptide inhibitors of sickle hemoglobin gelation. A study, in collaboration with the Clinical Hematology Branch, NHLBI, of the use of hydroxyurea, an inhibitor of DNA methylation which increases fetal hemoglobin levels, of the treatment of sickle cell anemia patients has continued.

The erythroid cell biology and molecular genetic studies have largely concentrated on the K562 human erythroleukemic cell line. It has been shown that hemin affects, in a reversible manner, the accumulation of various embryonic and fetal hemoglobin species in these cells. Recent results now show that this depends largely upon changes in the transcription of the specific globin genes. A "library" of K562 DNA has been constructed and the β-globin gene has been cloned from this. Using restriction enzymes, we have shown that its structure is normal. We have now transfected this cloned gene ligated into an expression vector into monkey kidney cells and have shown by S1 mapping that it is being transcribed normally. Thus, these results suggest that there are specific factors, trans-acting factors, in the K562 cell line which prevents transcription of the adult globin genes. In addition, the K562 cells are being used to develop a human cell "assay system" for the expression of the human globin genes. These cells have been transfected with a plasmid which contains the β-globin gene and the messenger RNA synthesized is being analyzed by molecular hybridization with the appropriate cDNA. Very recently we have initiated a study on the structure of transcriptionally active and inactive globin gene chromatin. These studies should clarify the control of globin gene synthesis in these cells and, possibly, all human erythroid cells.

Section on Protein Conformation

During the last year the efforts of this Section's work on protein folding and dynamics have centered on the study of cytochrome c. One project has as its goal the synthesis of cytochrome c by chemical and enzymatic means with the particular aim of synthesizing the ancestral cytochrome c of eukaryotes to learn of protein structure-function factors involved in the evolution of this gene which is central to all cell function. To this end chemical synthesis of three large fragments of the protein is being done, as is the isolation and characterization of the cytochrome c synthetase which forms the thioether bond to the heme. The enzyme has been solubilized and partially purified and has been used to attach the heme moiety to a synthetic fragment corresponding to residues 1-25. Chemical synthesis of other fragments, especially variants of fragment 28-38, is underway. Recently we have identified two enzymes in yeast and have solubilized the enzyme from beef heart mitochondria.

Another project has been the continued characterization of the dynamics of a three fragment non-covalent complex (with the heme attached to one fragment) of the protein. Several analogues of one of the fragments have been chemically synthesized and the dissociation constants of these complexes determined. The results indicate that modulation of the global cooperative interactions occur, without large conformational changes, between different energy states and are related to the mechanism of increasing the oxidation-reduction potential. The results indicate that the global cooperative interactions that stabilize the three-dimensional structure occur late in folding, not early as many kinetic models of folding assume. Thermal transition studies indicate that certain invariant residues determine global interactions as well as heme ligation. NMR studies are presently underway to measure the dynamics of these various fragment complexes.

Recently a new major project has been planned to study the specificity of antigen-antibody interactions. Monoclonal antibodies will be prepared to the fragment complexes of cytochrome c and the dynamics of antibody binding will be studied with respect to association and rate constants, including NMR measurements. Substitutions will be made chemically in the sequences of the cytochrom c fragments and genetic engineering will be used to make substitutions in the immunoglobulin sequences. This project will study the role of global cooperative interactions in antigen-antibody binding.

Section on Protein Chemistry

The research program in this Section concentrates on neurophysins and their interactions with oxytocin and vasopressin, as a model system for studying peptide-protein interactions in general. The neurophysin project has concentrated on attempting to identify granular proteases which cleave the spaces between the hormone and the carrier by using a synthetic substrate of neurophysin and oxytocin covalently linked. An enzymatic activity with some of the expected properties has been found. In addition, HPLC and radioimmunoassays are being used to identify neurophysin in non-brain tissues. Work on ribonuclease-S and thermolysin also continues.

SECTION ON MACROMOLECULAR BIOLOGY

I. Sickle Cell Anemia

A. The Intracellular Polymerization of Hemoglobin S

Hemoglobin composition and concentration are the primary determinants of polymer formation in erythrocytes containing sickle hemoglobin (SS). Polymer formation can be experimentally measured or calculated from a thermodynamic analysis. Polymer formation decreases with increasing oxygen saturation and polymer is present in vivo at high oxygen saturations (greater than 80% or 90%). Calculations of polymer formation which have now been extended to other sickle syndromes, based on the average hematologic profile, can explain to a large degree (about 80%) the variation in hemolytic severity associated with twelve different sickle syndromes.

The effects of cell heterogeneity within a sickle syndrome were examined for sickle cell anemia, with and without co-existing alpha-thalassemia, in terms of intracellular hemoglobin composition and hemoglobin concentration. The broad distribution in erythrocyte density, the large proportion of dense cells and the accelerated increase in cell density beyond normal cell aging in sickle cell anemia are reduced with coexisting alpha-thalassemia. The hemoglobin F production, reflected in the percentage of hemoglobin F containing reticulocytes and the proportion of hemoglobin F per F-cell remains unchanged.

Studies of intracellular hemoglobin composition and concentration are being used to evaluate therapeutic strategies. For example, 5-azacytidine and hydroxyurea treatment increase hemoglobin F production while decreasing hemoglobin S production. The effect of these treatments on cell density is being monitored to determine changes in the polymerization potential of the sickle cell. Studies of non-covalent modifiers of gelation are continuing with x-ray crystallographic analysis and energy minimization calculations to determine experimentally and theoretically possible binding sites of peptides which might mimic an intermolecular binding region. These studies will be used to improve the specificity of these potential therapeutic agents. (Noguchi, Rodgers, Schechter)

B. The Rheological Effects of Intracellular Polymerization

The pathophysiology of sickle cell disease at the cellular and tissue level is poorly understood despite detailed information about the physical chemistry of hemoglobin S solutions. During the last few years we have been studying the intracellular polymerization of hemoglobin S and have detected polymer at high oxygen saturation. The present program is a multifaceted approach to study the rheological effects of polymer formation in red cells using electron microscopy, cell aggregation, cell filterability and cell viscosity measurements.

We are using electron microscopy to study the amount and physical properties of the polymer of deoxyhemoglobin S that forms in sickle erythrocytes at various oxygen saturation values. The electron microscopic studies are being correlated with results from nuclear magnetic resonance spectroscopy and other methods to examine the physical, physiological and clinical variables that affect intracellular polymerization of deoxyhemoglobin S. These studies will allow us to test various hypothesis about the pathophysiology of sickle cell disease, specifically the relationship of the amount and length of the polymer to changes in cell rheology. These direct studies of polymer have been correlated with aggregation, filterability, and viscosity measurements in an attempt to understand how the amount of polymer and the rate of its formation impedes the flow of blood in the microcirculation of sickle cell patients. To facilitate these studies we have worked with BEIB to develop a filtration apparatus which allows us to measure deformability properties at various oxygen saturation values. This apparatus has allowed us to measure precisely several of the variables that determine the flow properties of sickle erythrocytes. We will also try to correlate disease severity with these rheological measurements. (Acquaye and Schechter)

C. The Development of Non-Invasive Methods to Assess Sickle Cell Patients

Although the pathophysiologic manifestations of sickle cell disease has been assumed to result from vaso-occlusion secondary to the rheologically abnormal erythrocytes, actual blood vessel obstruction has been rarely demonstrated *in vivo*. With the development of new non-invasive imaging and para-imaging methods, it is now technically possible and feasible to characterize both regional organ perfusion and tissue biochemistry in quantitative terms, and in addition attempt to reconstruct the pathophysiologic events responsible for initiation, ischemia, infarction and resolution in these patients. Our goals are to develop ways to examine the pathophysiological processes in various organs, to develop quantitative ways to assess disease severity and progression, and to develop objective means to evaluate potential therapeutic approaches. In preliminary analyses of anaerobic threshold measurements we have found that the vasodilator, nifedipine, increases the time of exercise before the occurrence of sustained anaerobiosis. After calibrating an analytical method to derive the distribution of intracellular hemoglobin concentrations present in the peripheral blood of sickle cell patients, we were able to demonstrate that the pathognomonic conjunctival "comma" sign relates to the extent of the red cell heterogeneity. Using the technique of laser-Doppler velocimetry, we have found that forearm cutaneous microcirculatory flow undergoes a unique characteristic periodic pattern. Preliminary studies of 2-deoxy-18 fluoro-deoxyglucose brain metabolism suggest that there are regional and hemispheric topographical abnormalities when compared to age-matched controls. Feasibility studies are currently underway to determine the utility of nuclear magnetic resonance imaging in these patients. These studies should facilitate clinical trials of therapeutic agents designed to maintain or improve the biological integrity of jeopardized organs in patients with sickle cell disease. We plan to extend these studies to patients with other sickle cell syndromes. (Rodgers, Noguchi, and Schechter)

II. Erythroid cell Biology and Molecular Genetics

A. Regulation of Hemoglobin Synthesis in Cultured Erythroid Cells

We are studying control of globin gene expression in K562 human leukemia cells which, we have shown, synthesize embryonic and fetal hemoglobins, but not adult hemoglobin A, free β-chains, or β-globin RNA. The karyotype of this transformed cell line demonstrates triploidy with a translocation of varying length on the distal end of the short arm of one chromosome 11 (11p), near the locations proposed for the ε-γ-β-globin gene complex, as well as the insulin gene and the c-Ha-ras-1 protooncogene. Using *in situ* hybridization of tritium-labelled probes to K562 metaphase chromosomes, we localized these five genes to the proximal one-third of chromosome 11p. Since this localization differed from several reports, we performed extensive *in situ* hybridization studies with normal lymphocytes and showed that the ε-γ-β-globin genes are located at somatic metaphase bands 11.2-12, while the ras and insulin genes are at band 14.1, identical to the results obtained with K562 cells. The results suggest these genes are distant from the K562 translocation breakpoint.

To investigate whether DNA methylation was related to the lack of expression of the K562 β-globin gene we cultured cells in 5-azacytidine (5-AzaC), a cytidine analogue which causes hypomethylation of DNA. We detected, by S1 nuclease analysis, low but significant levels of β-globin mRNA following 5-AzaC treatment, while other globin mRNAs increased in abundance. The DNA of K562 cells became severely hypomethylated. However, the methylation-sensitive restriction enzyme HpaII revealed very low levels of modification at most of its recognition sites within the globin gene locus prior to treatment, and no further demethylation following treatment. Furthermore, in studies with hydroxyurea (HU)and cytosine-arabinoside, two other S-phase specific cytostatic/cytotoxic drugs, we have also observed induction of globin gene synthesis in K562 cells, and, with HU, expression of β-globin mRNA. The results suggest that these three drugs may act, at least in part, as chemical modifiers which affect the regulatory environment within cells, including trans-acting factors. (Dean, Fordis, Wu, Flanagan, and Schechter)

B. Analysis of Globin Gene Expression in K562 Human Leukemia Cells

The K562 human leukemia cell is an erythroid-like cell that may serve as a model for the study of globin gene expression in transcriptionally active human erythroid cells. K562 cells express all globin genes with the exception of that for β-globin; failure to produce β-globin could result from an acquired mutation in each of the β-globin genes or from an alteration in the regulatory factor environment of the β-globin gene. To uncover a possible acquired mutation, restriction endonuclease analysis of genomic K562 DNA and expression studies of a cloned K562 β-globin genes were carried out. Analysis of the polymorphic Ava II site in intervening sequence 2 of the β-globin gene showed that K562 cells contain two different β-globin alleles, both of which are inactive.' A K562 β-globin gene was cloned, ligated into the expression vector pLTN3B, and introduced into COS cells. Transcripts were analyzed by RNA blot, dot blot, S1 nuclease digestion, and primer extension assay. The cloned K562 β-globin gene was transcribed in COS cells as efficiently as a normal β-globin gene introduced into COS cells; the mRNA was 10 S and polyadenylated; the 5' and 3' termini and the processing of transcripts were identical to that of mRNA transcribed from a normal gene. Based on these data we suggest that the absence of β-globin gene expression results not from an alteration in the β-globin gene, but from a quantitative or qualitative alteration in a trans-acting factor important in β-globin gene expression.

In additional experiments, methods have been developed to reproducibly transfer with high efficiency exogeneous genes into K562 cells. When a β-globin promoter fused to a bacterial gene is introduced into K562 cells no expression is seen, a result consistent with our findings above. Using this observation, techniques for isolating the gene(s) for the trans-acting factors are being developed. Furthermore, with the transformation procedure globin promoters (or deletion mutations thereof) fused to bacterial marker genes are being introduced into K562 cells to study cis-acting sequences important in hemin induction and the tissue specificity of expression. (Fordis, Nelson, Dean, and Schechter)

C. Chromatin Structure and Transcriptional Activation in K562 Human Leukemia Cells.

The K562 cell is being studied as a model of human globin gene expression. K562 cells express embryonic and fetal hemoglobins but not hemoglobin A, β-globin chains, nor β-globin mRNA. Understanding the pattern of globin expression in K562 cells may yield insight into globin expression and hemoglobin switching in normal cells. Previous work on the integrity of the β-globin gene has been extended to show that chromosomal alterations do not account for the pattern of β-like globin expression seen in K562 cells. In addition, in situ hybridization has been used to assign the β-globin gene locus to the chromosome region 11p11.2 to 12. Presently, studies are underway to correlate the observed pattern of sensitivity and hypersensitivity to DNase I digestion to possible alterations in chromosome structure in the transcriptionally active β-globin gene. This is being done by sedimentation analysis of chromatin fragments. Additionally, future experiments are planned to footprint non-histone proteins binding to the flanking regions of active globin genes. These proteins are putative trans-acting factors which may have a causal relationship to gene transcription. (Flanagan, Dean, Fordis, and Schechter)

D. Induction of Differentiation in K562 Human Leukemia Cells

We have been studying the K562 human leukemia cell line in order to analyze the control of human erythroid differentiation and globin gene expression. We have shown that hemin causes induction of hemoglobin synthesis by increasing mRNA levels and that this process is fully reversible, without evidence of terminal differentiation.

We have studied the physiological properties of the embryonic and fetal hemoglobins synthesized in these cells as a response to hemin. We find that following induction of hemoglobin synthesis K562 cells demonstrate oxygen dissociation properties similar to human embryonic red cells. The P50 value of the hemoglobin is 20 ± 0.9 mmHg. The Hill coefficient is 2.5. Intracellular pH of K562 cells, measured by 31-P NMR, is 7.3. We also find that the levels of 2,3-diphosphoglycerate (DPG), a potent modifier of hemoglobin function, increase dramatically upon the induction of hemoglobin synthesis in K562 cells. In a typical experiment, DPG levels went from 8 nmoles/10E8 cells to 64 nmoles/10E8 cells upon induction of hemoglobin. For several experiments there was an approximately linear relationship between DPG and hemoglobin levels. The coordinate increase in DPG and hemoglobin levels has been demonstrated not only by an enzymatic method but also by 31-P NMR spectroscopy. These data suggest that DPG and hemoglobin levels are coordinately controlled in these cells. We are now investigating the molecular mechanism of the coordinate increase in DPG and hemoglobin levels in response to hemin induction.

In more recent experiments we have examined the effects of various analogs of 5-azacytidine which may also inhibit DNA methylation for their ability to induce hemoglobin synthesis in K562 cells. We find that 3-deazadenosine and several other analogs do cause induction. We are examining the molecular basis of this phenomenon as it may be relevant to the treatment of sickle cell anemia and thalassemia patients in an effort to increase fetal hemoglobin synthesis. (Wu, Dean, and Schechter)

SECTION ON PROTEIN CONFORMATION

I. Evolution, Folding and Dynamics of Cytochrome c

A. Chemical Synthesis of Cytochrome c: Evolution of Cytochrome c

Cytochrome c is a key protein in the electron transfer system of eucaryotic mitochondria. Its amino acid sequence and structure are homologous after evolution time spanning more than a billion years. To investigate the role of individual amino acid residues on the structure and function, a method to replace an amino acid at any position of the amino acid sequence would be useful in substitution or labelling with isotope of individual amino acids. The method is a total synthesis of cytochrome c. As reported previously, a breakthrough came in an attempt of this synthesis when we found cytochrome c synthetase in yeast mitochondria. The enzyme catalyzes covalent bonding of heme to apocytochrome c through two thioether bonds.

In the current year, we found that there are two cytochrome c syntheases in yeast mitochondria, one solubilized, recognizing yeast and Candida krusei apocytochrome c and partially tuna apocytochrome c but not horse apocytochrome c and the other not solubilized, recognizing both yeast and horse apocytochromes c. We speculate that the latter less specific enzyme might catalyze also covalent attachment of heme to apocytochrome c_1. We have also solubilized cytochrome c synthetase from beef heart mitochondria and found that this solubilized enzyme can catalyze attachment of heme to either horse or yeast apocytochrome c. Thus, this enzyme may be used in synthesis of cytochrome c. (Taniuchi, Di Bello, and Visco)

B. Mechanism of Protein Folding: Extra Force Due to Coupling

The studies in the previous years has led to a hypothesis that the state of one region of the structures of proteins is coupled in a specific way with those of distant regions after folding of some unit of the amino acid sequence to generate extra force for stabilization of the structure.

To investigate the role of individual residues in this hypothetical coupling we have chemically synthesized 14 fragment analogs of residues 28 to 38 of cytochrome c as described in the previous year. The binding constants of these analogs with a two-fragment complex containing a heme fragment residues 1 to 25, (1-25)H, and an apofragment of residues 38 to 104, (38-104), to form a productive, three-fragment complex was determined as a function of temperature. The results have shown a) replacement of invariant proline 30, leucine 32 or glycine 34 profoundly affects the enthalpy-entropy compensation involved in the binding, resulting in destabilization while the effect of substitution of partially invariant leucine 35 with isoleucine is small; b) hydrophobic interaction is not primarily responsible for the destabilization. The thermal transition of the absorption band at 695 nm and kinetics of this transition or the fragment exchange of the complex have indicated that substitution of some of invariant residues (e.g. leucine 32, glycine 34) would affect not only the interatomic interactions at the substituted sites but also those involved in ligation of methionine 80 to the heme iron or concerted global motion. Thus, the invariance of leucine 32 and glycine 34 may be related to the coupling phenomenon. There may also be a link between the enthalpy-entropy compensation phenomenon known for protein folding and the mechanisms of the coupling.

Our aim is to find the mechanism of the coupling which underlies the structure, function and folding of proteins. (Taniuchi, Poerio, Roy, and Parr)

II. Immunoglobulin Dynamics

A. Origin of Specificity of Antigen-Antibody Interaction

It is generally considered that spatial complementarity would determine the specificity of antigen-antibody interactions. On the other hand, it is also reported that substitution of amino acids in a distant region from the binding site profoundly affects the antigen-antibody interaction (Cook, W.D. et al., (1982) P.N.A.S. 79, 1240). Such mutual influence between distant regions is very characteristic of the hypothetical global cooperative interactions maintaining the three-dimensional structure of proteins (see the other reports from this Section). Thus the two phenomena might be closely related. Consistent with this view, studies using ^1H-^2H exchange, show that a polyclonal antibody exhibits a reduced motility and an increased stability without a large change in conformation upon binding with a hapten (Zavidszky, P., et al. (1981) Mol. Immunol. 18, 39). Thus, we propose to investigate the specificity of antigen-antibody interaction in relation to the global cooperative interactions as follows:

1) A three-fragment complex of yeast cytochrome c will be developed. 2) A monoclonal antibody will be prepared to yeast cytochrome c. Then, the antigenic site will be determined using the fragment complex. 3) Thermodynamic and kinetic parameters of the antibody-antigen reaction with the fragment complex will be determined as a function of substitution of amino acids of the antigen using chemical synthesis. 4) Change in molecular motion of antibody upon binding with antigen containing unsubstituted and substituted amino acids will be determined using ^1H-^3H or H-^2H exchange. The degree of these changes will be related to the strength of binding with antigen. 5) A fragment exchange technique will also be used to investigate global influence on the antibody of antigen-antibody interaction. 6) Gene manipulation will be contemplated to investigate the effect of substitution of amino acids of the variable domain of light chains (and heavy chains, if possible) on the antigen-antibody interaction. (Taniuchi)

SECTION ON PROTEIN CHEMISTRY

I. Structural Bases of Biological Function of Peptides and Proteins

A. Model Systems

Principles which govern conformation, surface recognition and function of peptides and proteins have been studied for neurophysin-neuropeptide hormone complexes and their precursors as well as for several related peptides and proteins. Molecular recognition by peptides and proteins underlies essentially all biological functions of these substances, emphasizing the importance of understanding surface organization and dynamics in defining structural bases of function. This issue addressed for the neurohypophysial hormones oxytocin and vasopressin and associated neurophysins, which form cooperative peptide-protein complexes that act as storage forms for the polypeptides in neurosecretory granules. The nature and structural interrelationships between the self-association and hormone binding surfaces in neurophysins that give rise to cooperative complexes (marked by quaternary enhancement) have been studied. Methods for analytical high performance affinity chromatography are being developed for the evaluation of these interactions for molecular species isolated both from various neuroendocrine sites and by in vitro sequence modification. Neurophysin isoforms of related but variant sequences have been identified and shown to have similar hormonal recognition properties in spite of sequence changes. Separately, the concept of interdependence between structural framework and surface recognition of peptides and proteins has been evaluated by studying the effect of synthetic sequence modeling of ribonuclease S-peptide on forming functional ribonuclease-S. The structure of a modeled semisynthetic ribonuclease-S is being solved to 3A° resolution by high resolution X-ray diffraction analysis. In addition, the way in which intramolecular domains participate in folding and stabilization continues to be evaluated in a study of large, C-terminal-derived, fragments of thermolysin. A role is suggested for domains in forming intramolecular inter-domain surface contacts to stabilize overall native protein structure. The role of domains in folding per se is being evaluated. (Chaiken, Swaisgood, Kanmera, and Sequeira)

B. Neuroendocrine Peptides and Proteins

Molecular mechanisms underlying the neuroendocrine occurrence and function of neurophysins (NP's) and associated neuropeptide hormones oxytocin and vasopressin have been studied. The NP's and neuropeptides are dominant polypeptides of the hypothalamo-neurohypophysial system ("classical" site of occurrence). Their intraneuronal biosynthesis, translocation, and structural transitions from precursors to mature, interacting polypeptides comprise major elements of neurosecretory functions. NP and neuropeptide hormone-related polypeptides also have been detected in putative neuromodulator-neurotransmitter pathways within the central nervous system as well as in several peripheral (e.g. endocrine) sites ("non-classical" sites of occurrence). Separately, an hypothesis has been developed and is being examined that biosynthetic precursors of the neurohypophysial hormones have a defined conformational organization and that this organization helps regulate the production of active peptides produced in neuroendocrine pathways which make the precursors. A method was devised to produce biosynthetic precursor, and ultimately sequence modelled and site-specific mutants; a semisynthetic oxytocin/neurophysin precursor analog has been made. An evaluation of structural characteristics of the semisynthetic precursor, and the impact of these characteristics on enzymatic processing reactions, is in progress. During the current year, a study has been continued of "non-classical" sites of the hormone/NP neuroendocrine system, including the ovary and peripheral neurons. Both hormone-and NP-immunoreactive molecules have been found in the ovary. The results so far that the ovary may be a site of local synthesis of both oxytocin-, vasopressin-, and neurophysin-related molecules. The relationships of ovarian molecular species to those produced in the hypothalamo-neurohypophysial pathway are being studied. The molecular mechanisms and neuroendocrine peptide function in the ovary, as in many other "non-classical" sites, remains to be defined rigorously. (Chaiken, Kanmera, and Sequeira)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01AM25,003-14 LCB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structural Bases of Biological Function of Peptides and Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Irwin M. Chaiken	Research Chemist	LCB, NIADDK
Others:	Harold Swaisgood	IPA Appointee & Guest Worker	LCB, NIADDK
	Tatsuhiko Kanmera	Visiting Fellow	LCB, NIADDK
	Reginald Sequeira	Visiting Fellow	LCB, NIADDK

COOPERATING UNITS *(if any)*
Biochemistry Dept., Duke University, Durham, NC; Institute of Organic Chemistry, University of Padova, Padova, Italy; Chem. Dept., Univ. of Maryland, College Park, MD; Biology Dept., Johns Hopkins University, Baltimore, MD

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Protein Chemistry

INSTITUTE AND LOCATION
NIADDK, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.4	1.2	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Principles which govern conformation, surface recognition and function of peptides and proteins have been studied for neurophysin-neuropeptide hormone complexes and their precursors as well as for several other advantagous peptides and proteins. Molecular recognition by peptides and proteins underlies essentially all biological functions of these substances, emphasizing the importance of understanding surface organization and dynamics in defining structural bases of function. This issue has been addressed for the neurohypophysial hormones oxytocin and vasopressin and associated neurophysins, which form cooperative peptide-protein complexes that act as storage forms for the polypeptides in neurosecretory granules. The nature and structural interrelationships between the self-association and hormone binding surfaces in neurophysins that give rise to cooperative complexes (marked by quaternary enhancement) have been studied. Methods for analytical high performance affinity chromatography are being developed for the evaluation of these interactions for molecular species isolated both from various neuroendocrine sites and by in vitro sequence modification. Neurophysin isoforms of related but variant sequences have been identified and shown to have similar hormonal recognition properties in spite of sequence changes. Separately, the concept of interdependence between structural framework and surface recognition of peptides and proteins has been evaluated by studying the effect of synthetic sequence modeling of ribonuclease S-peptide on formation of functional ribonuclease-S. The structure of a modeled semisynthetic ribonuclease-S is being solved to high resolution by X-ray diffraction analysis. In addition, the way in which intramolecular domains participate in folding and stabilization continues to be evaluated in a study of large, C-terminal-derived, fragments of thermolysin. A role is suggested for domains in forming intramolecular inter-domain surface contacts to stabilize overall native protein structure. The role of domains in protein folding per se is being evaluated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01AM25,007-11 LCB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Neuroendocrine Peptides and Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Irwin M. Chaiken	Research Chemist	LCB, NIADDK
Other:	Tatsuhiko Kanmera	Visiting Fellow	LCB, NIADDK
	Reginald Paul Sequeira	Visiting Fellow	LCB, NIADDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Protein Chemistry

INSTITUTE AND LOCATION
NIADDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
3.3	2.5	.8

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Molecular mechanisms underlying the neuroendocrine occurrence and function of neurophysins (NP's) and associated neuropeptide hormones oxytocin and vasopressin have been studied. The NP's and neuropeptides are dominant polypeptides of the hypothalamo-neurohypophysial system ("classical" site of occurrence). Their intraneuronal biosynthesis, translocation, and structural transitions from precursors to mature, interacting polypeptides comprise major elements of neurosecretory function. NP and neuropeptide hormone-related polypeptides also have been detected in putative neuromodulator-neurotransmitter pathways within the central nervous system as well as in several peripheral (e.g. endocrine) sites ("non-classical" sites of occurrence). During the current year, a study has been continued of "non-classical" sites of the hormone/NP neuroendocrine system, including the ovary and peripheral neurons. Both hormone- and NP-immunoreactive molecules have been found in the ovary. The results so argue far that the ovary may be a site of local synthesis of both oxytocin-, vasopressin-, and neurophysin-related molecules. The relationships of ovarian molecular species to those produced in the hypothalamo-neurohypophysial pathway are being studied. Some of our recent results suggest that structurally unique neuroendocrine peptides are produced in different pathways. The molecular mechanisms and neuroendocrine peptide function in the ovary, as in many other "non-classical" sites, remains to be defined rigorously. Separately, an hypothesis has been developed and is being examined that biosynthetic precursors of the neurohypophysial hormones have a defined conformational organization and that this organization helps regulate the production of active peptides produced in neuroendocrine pathways which make the precursors. A method was devised to produce biosynthetic precursor, and ultimately sequence modeled and site-specific mutants; a semisynthetic oxytocin/neurophysin precursor analog has been made. An evaluation of structural characteristics of the semisynthetic precursor, and the impact of these characteristics on enzymatic processing reactions, is in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AM25,008-21 LCB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chemical Synthesis of Cytochrome c: Evolution of Cytochrome c

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Hiroshi Taniuchi, Chief, Section on Protein Conformation, LCB, NIADDK

Other:	Carlo Di Bello	Visiting Scientist	LCB, NIADDK
	Carlo Visco	Visiting Fellow	LCB, NIADDK

COOPERATING UNITS *(if any)*
University of Padova, Padova, Italy

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Laboratory of Chemical Biology

SECTION
Section on Protein Conformation

INSTITUTE AND LOCATION
NIADDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.7	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cytochrome c is a key protein in the electron transfer system of eucaryotic mitochondria. Its amino acid sequence and structure are homologous after evolution time spanning more than a billion years. To investigate the role of individual amino acid residues on the structure and function, a method to replace an amino acid at any position of the amino acid sequence would be useful in substitution or labelling with isotope of individual amino acids. The method is a total synthesis of cytochrome c. As reported previously, a breakthrough came in an attempt of this synthesis when we found cytochrome c synthetase in yeast mitochondria. The enzyme catalyzes covalent bonding of heme to apocytochrome c through two thioether bonds.

In the current year, we found that there are two cytochrome c syntheases in yeast mitochondria, one solubilized, recognizing yeast and Candida krusei apocytochrome c and partially tuna apocytochrome c but not horse apocytochrome c and the other not solubilized, recognizing both yeast and horse apocytochromes c. We speculate that the latter less specific enzyme might catalyze also covalent attachment of heme to apocytochrome c_1. We have also solubilized cytochrome c synthetase from beef heart mitochondria and that this solubilized enzyme can catalyze attachment of heme to either horse or yeast apocytochrome c. Thus, this enzyme may be used in synthesis of cytochrome c.

As a first step of synthesis of horse cytochrome c apofragment containing residues 1 to 37 was synthesized using the Merrifield's improved solid phase method and is being purified to be tested as a substrate for cytochrome c synthetase. An undecapeptide containing residues 28 to 36 plus methonine and glycine as the penultimate and COOH-terminal residues, respectively was also synthesized to be linked to a semisynthetic apofragment of residues 38 to 104. A fragment of residues 66 to 104 is also being synthesized to be linked with fragment residues 28 to 65. These fragments would be the building blocks in the total synthesis of cytochrome c.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01AM25,011-10 LCB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanism of Protein Folding: Extra Force Due to Coupling

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Hiroshi Taniuchi, Chief, Section on Protein Conformation, LCB, NIADDK

Others:	Elia Poerio	Visiting Scientist	LCB, NIADDK
	Siddhartha Roy	Visiting Associate	LCB, NIADDK
	Gary R. Parr	Research Chemist	LCB, NIADDK

COOPERATING UNITS *(if any)*
Biophysical Pharmacology Section, Clinical Pharmacology Branch, NCI; Department of Biochemistry, Clemson University, Clemson, SC; Department of Biochemistry, University of Geneva, Geneva Switzerland

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Laboratory of Chemical Biology

SECTION
Section on Protein Conformation

INSTITUTE AND LOCATION
NIADDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The studies in the previous years has led to a hypothesis that the state of one region of the structures of proteins is coupled in a specific way with those of distant regions after folding of some unit of the amino acid sequence to generate extra force for stabilization of the structure.

To investigate the role of individual residues in this hypothetical coupling we have chemically synthesized 14 fragment analogs of residues 28 to 38 of horse cytochrome c as described in the previous year. The binding constants of these analogs with a two-fragment complex containing a heme fragment residues 1 to 25 and an apofragment of residues 38 to 104 to form a productive, three-fragment complex was determined as a function of temperature. The results have shown a) replacement of invariant proine 30, leucine 32 or glycine 34 profoundly affects the enthalpy-entropy compensation involved in the binding, resulting in destabilization while the effect of substitution of partially invariant leucine 35 with isoleucine is small; b) hydrophobic interaction is not primarily responsible for the destabilization. The thermal transition of the absorption band at 695 nm and kinetics of this transition or the fragment exchange of the complex have indicated that substitution of some of invariant residues (e.g. leucine 32, glycin 34) would affect not only the interatomic itneractions at the substituted sites but also those involved in ligation of methionine 80 to the heme iron or a concerted global motion. Thus, the invariance of leucine 32 and glycine 34 may be related to the coupling phenomenon. There may also be a link between the enthalpy-entropy compensation phenomenon known for protein folding and the mechanisms of the coupling.

Our aim is to find the mechanism of the coupling which underlies the structure, function and folding of proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01AM25,016-11 LCB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Hemoglobin Synthesis in Cultured Erythroid Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ann Dean	Research Chemist	LCB, NIADDK
Others:	C. Michael Fordis	Medical Staff Fellow	LCB, NIADDK
	Yongji Wu	Visiting Fellow	LCB, NIADDK
	Margaret Flanagan	Guest Worker (NRSA Fellow)	LCB, NIADDK
	Alan N. Schechter	Chief	LCB, NIADDK

COOPERATING UNITS *(if any)* Clinical Hematology Branch, NHLBI (Dr. T. Ley); Div. of Cancer Cure and Prevention, NCI (Dr. V. Wilson); Medicine Branch, NCI (Dr. J. Whang-Peng and Ms. T. Knudsen); Lab. of General and Comparative Biochemistry, NIMH (Dr. G. Cantoni)

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Laboratory of Chemical Biology

SECTION
Section on Macromolecular Biology

INSTITUTE AND LOCATION
NIADDK, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.3	1.2	0.1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are studying control of globin gene expression in K562 human leukemia cells which, we have shown, synthesize embryonic and fetal hemoglobins, but not adult hemoglobin A, free β-chains, or β-globin RNA. The karyotype of this transformed cell line demonstrates triploidy with a translocation of varying length on the distal end of the short arm of one chromosome 11 (11p), near the locations proposed for the ε-γ-β-globin gene complex, as well as the insulin gene and the c-Ha-ras-1 protooncogene. Using in situ hybridization of tritium-labelled probes to K562 metaphase chromosomes, we localized these five genes to the proximal one-third of chromosome 11p. Since this localization differed from several reports, we performed extensive in situ hybridization studies with normal lymphocytes and showed that the ε-γ-β-globin genes are located at somatic metaphase bands 11.2-12, while the ras and insulin genes are at band 14.1, identical to the results obtained with K562 cells. The results suggest these genes are distant from the K562 translocation breakpoint.

To investigate whether DNA methylation was related to the lack of expression of the K562 β-globin gene we cultured cells in 5-azacytidine (5-AzaC), a cytidine analogue which causes hypomethylation of DNA. We detected, by S1 nuclease analysis, low but significant levels of β-globin mRNA following 5-AzaC treatment, while other globin mRNAs increased in abundance. The DNA of K562 cells became severely hypomethylated. However, the methylation-sensitive restriction enzyme HpaII revealed very low levels of modification at most of its recognition sites within the globin gene locus prior to treatment, and no further demethylation following treatment. Furthermore, in studies with hydroxyurea (HU) and cytosine-arabinoside, two other S-phase specific cytostatic/cytotoxic drugs, we have also observed induction of globin gene synthesis in K562 cells, and, with HU, expression of β-globin mRNA. The results suggest that these three drugs may act, at least in part, as chemical modifiers which affect the regulatory environment within cells, including trans-acting factors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01AM25,021-09 LCB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Sickle Cell Anemia: The Intracellular Polymerization of Hemoglobin S

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Constance T. Noguchi	Senior Staff Fellow	LCB, NIADDK
Others:	Griffin P. Rodgers	Guest Worker (Robert Wood Johnson Fellow)	LCB, NIADDK
	Alan N. Schechter	Chief	LCB, NIADDK

COOPERATING UNITS *(if any)* CHB, NHLBI (Drs. A.W. Nienhuis; T. Ley); BB, CC (Dr. H. Klein); Case-Western Res. (Dr. G. Brittenham); Johns Hopkins (Dr. G.N. Dover); Cornell (Dr. H. Scheraga); Univ. West Indies (Dr. G. Serjeant); M.R.C. Lab., Oxford (Drs. D.J. Weatherall; D.R. Higgs), M.R.C. Lab., Cambridge (Dr. M.F. Perutz).

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Macromolecular Biology

INSTITUTE AND LOCATION
NIADDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Hemoglobin composition and concentration are the primary determinants of polymer formation in erythrocytes containing sickle hemoglobin (SS). Polymer formation can be experimentally measured or calculated from a thermodynamic analysis. Polymer formation decreases with increasing oxygen saturation and polymer is present in vivo at high oxygen saturations (greater than 80% or 90%). Calculations of polymer formation which have now been extended to other sickle syndromes, based on the average hematologic profile, can explain to a large degree (about 80%) the variation in hemolytic severity associated with twelve different sickle syndromes. The effects of cell heterogeneity within a sickle syndrome were examined for sickle cell anemia, with and without co-existing alpha-thalassemia, in terms of intracellular hemoglobin composition and hemoglobin concentration. The broad distribution in erythrocyte density, the large proportion of dense cells and the accelerated increase in cell density beyond normal cell aging in sickle cell anemia are reduced with coexisting alpha-thalassemia. The hemoglobin F production, reflected in the percentage of hemoglobin F containing reticulocytes and the proportion of hemoglobin F per F-cell remains unchanged. Studies of intracellular hemoglobin composition and concentration are being used to evaluate therapeutic strategies. For example, 5-azacytidine and hydroxyurea treatment increase hemoglobin F production while decreasing hemoglobin S production. The effect of these treatments on cell density is being monitored to determine changes in the polymerization potential of the sickle cell. Studies of non-covalent modifiers of gelation are continuing with x-ray crystallographic analysis and energy minimization calculations to determine experimentally and theoretically possible binding sites of peptides which might mimic an intermolecular binding region. These studies will be used to improve the specificity of these potential therapeutic agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01AM25,023-08 LCB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Surface Molecules in Cell Recognition and Communication

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Edward Steers, Jr.	Research Biologist	LCB, NIADDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemical Biology

SECTION

Section on Protein Chemistry

INSTITUTE AND LOCATION

NIADDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01AM25,025-08 LCB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Origin of specificity of antigen-antibody interaction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI: Hiroshi Taniuchi, Chief, Section on Protein Conformation, LCB, NIADDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Protein Conformation

INSTITUTE AND LOCATION
NIADDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

It is generally considered that spatial complementarity would determine the specificity of antigen-antibody interactions. On the other hand, it is also reported that substitution of amino acids in a distant region from the binding site profoundly affects the antigen-antibody interaction (Cook, W.D. et al., (1982) P.N.A.S. 79, 1240). Such mutual influence between distant regions is very characteristics of the hypothetical global cooperative interactions maintaining the three-dimensional structure of proteins (see the other reports from this Section). Thus the two phenomena might be closely related. Being consistent with this view, studies using ^{1}H-^{2}H exchange, show that a polyclonal antibody exhibits a reduced motility and an increased stability without a large change in conformation upon binding with a hapten (Zavidszky, P., et al. (1981) Mol. Immunol. 18, 39). Thus, we propose to investigate the specificity of antigen-antibody interaction in relation to the global cooperative interactions as follows.

1) A three-fragment complex of yeast cytochrome c will be developed. 2) A monoclonal antibody will be prepared to yeast cytochrome c. Then, the antigenic site will be determined using the fragment complex. 3) Thermodynamic and kinetic parameters of the antibody-antigen reaction with the fragment complex will be determined as a function of substitution of amino acids of the antigen using chemical synthesis. 4) Change in molecular motion of antibody upon binding with antigen containing unsubstituted and substituted amino acids will be determined using ^{1}H-^{3}H or H-^{2}H exchange. The degree of these changes will be related to the strength of binding with antigen. 5) A fragment exchange technique will also be used to investigate global influence on the antibody of antigen-antibody interaction. 6) Gene manipulation will be contemplated to in-vestigate the effect of substitution of amino acids of variable domain of light chains (and heavy chain, if possible) on the antigen-antibody interaction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01AM25,028-06 LCB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Development of Non-Invasive Methods to Assess Sickle Cell Patients

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Griffin P. Rodgers	Guest Worker	LCB, NIADDK
Others:	Constance T. Noguchi	Senior Staff Fellow	LCB, NIADDK
	Alan N. Schechter	Chief	LCB, NIADDK

COOPERATING UNITS *(if any)*
Clinical Hematology Branch, NHLBI (Dr. A.W. Nienhuis); Clinical Branch, NEI (Dr. M. Roy); Blood Bank, CC (Dr. H. Klein); Nuclear Medicine, CC (Dr. R. Kessler); DCRT (Dr. R. Burgess); BEIB (Dr. R. Bonner).

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Macromolecular Biology

INSTITUTE AND LOCATION
NIADDK, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
1.1	1.1	

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Although the pathophysiologic manifestations of sickle cell disease has been assumed to result from vaso-occlusion secondary to the rheologically-abnormal erythrocytes, actual blood vessel obstruction has been rarely demonstrated in vivo. With the development of new non-invasive imaging and para-imaging methods, it is now technically possible and feasible to characterize both regional organ perfusion and tissue biochemistry in quantitative terms, and in addition attempt to reconstruct the pathophysiologic events responsible for initiation, ischemia, infarction and resolution in these patients. Our goals are to develop ways to examine the pathophysiological processes in various organs, to develop quantitative ways to assess disease severity and progression, and to develop objective means to evaluate potential therapeutic approaches. In preliminary analyses of anaerobic threshold measurements we have found that the vasodilator, nifedipine, increases the time of exercise before the occurrence of sustained anaerobiosis. After calibrating an analytical method to derive the distribution of intracellular hemoglobin concentrations present in the peripheral blood of sickle cell patients, we were able to demonstrate that the pathognomonic conjunctival "comma" sign relates to the extent of the red cell heterogeneity. Using the technique of laser-Doppler velocimetry, we have found that forearm cutaneous microcirculatory flow undergoes a unique characteristic periodic pattern. Preliminary studies of 2-deoxy-18 fluoro-deoxyglucose brain metabolism suggest that there are regional and hemispheric topographical abnormalities when compared to age-matched controls. Feasibility studies are currently underway to determine the utility of nuclear magnetic resonance imaging in these patients. These studies should facilitate clinical trials of therapeutic agents designed to maintain or improve the biological integrity of jeopardized organs in patients with sickle cell disease. We plan to extend these studies to patients with other sickle cell syndromes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01AM25,029-06 LCB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Early Mammalian Development: Biosynthesis of the Zona Pellucida Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jurrien Dean	Senior Investigator	LCB, NIADDK
Others:	Iain East	Visiting Fellow	LCB, NIADDK

COOPERATING UNITS *(if any)*
Nagoya University, Japan (Dr. Masahiro Tsuji)
Aichi Cancer Center Research Institute, Japan (Dr. Satoru Shimizu)

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Macromolecular Biology

INSTITUTE AND LOCATION
NIADDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
Transferred to Laboratory of Cellular and Developmental Biology, NIADDK

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM25,034-05 LCB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Early Mammalian Development: Processing of Sperm Nuclei by Oocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jurrien Dean	Senior Investigator	LCB, NIADDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Macromolecular Biology

INSTITUTE AND LOCATION
NIADDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Transferred to Laboratory of Cellular and Developmental Biology, NIADDK

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01AM25,038-04 LCB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Analysis of Globin Gene Expression in K562 Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C.M. Fordis	Medical Staff Fellow	LCB, NIADDK
Others:	N. Nelson	Chemist	LCB, NIADDK
	A. Dean	Research Chemist	LCB, NIADDK
	A.N. Schechter	Chief	LCB, NIADDK

COOPERATING UNITS *(if any)*
Clinical Hematology Branch, NHLBI (Drs. A.W. Nienhuis and N.P. Anagnou); Laboratory of Molecular Biology, NCI (Drs. B. Howard, C. Gorman, M. McCormick, and R. Padamanablan).

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Macromolecular Biology

INSTITUTE AND LOCATION
NIADDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	1.0	0.9

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The K562 human leukemia cell is an erythroid-like cell that may serve as a model for the study of globin gene expression in transcriptionally active human erythroid cells. K562 cells express all globin genes with the exception of that for β-globin; failure to produce β-globin could result from an acquired mutation in each of the β-globin genes or from an alteration in the regulatory factor environment of the β-globin gene. To uncover a possible acquired mutation, restriction endonuclease analysis of genomic K562 DNA and expression studies of a cloned K562 β-globin genes. Analysis of the polymorphic Ava II site in intevening sequence 2 of the β-globin gene showed that K562 cells contain two different β-globin alleles, both of which are inactive. A K562 β-globin gene was cloned, ligated into the expression vector pLTN3B, and introduced into COS cells. Transcripts were analyzed by RNA blot, dot blot, S1 nuclease digestion, and primer extension assay. The cloned K562 β-globin gene was transcribed in COS cells as efficiently as a normal β-globin gene introduced into COS cells; the mRNA was 10 S and polyadenylated; the 5' and 3' termini and the processing of transcripts were identical to that of mRNA transcribed from a normal gene. Based on these data we suggest that the absence of β-globin gene expression results not from an alteration in the β-globin gene, but from a quantitative or qualitative alteration in a trans-acting factor important in β-globin gene expression.

In additional experiments, methods have been developed to reproducibly transfer exogeneous genes into K562 cells. When a β-globin promoter fused to a bacterial gene is introduced into K562 cells no expression is seen, a result consistent with our findings above. Using this observation, techniques for isolating the gene(s) for the trans-acting factors are being developed. In other experiments cis-acting sequences important in human globin induction and the tissue specificity of expression are being studied.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM25,039-03 LCB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Monoclonal Antibodies as Probes to the Murine Zona Pellucida

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Iain East	Visiting Fellow	LCB, NIADDK
Other:	Jurrien Dean	Senior Investigator	LCB, NIADDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Macromolecular Biology

INSTITUTE AND LOCATION
NIADDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Transferred to Laboratory of Cellular and Developmental Biology, NIADDK

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01AM25,040-03 LCB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cell-Cell Interactions: Attachment of Paramecium to Polystyrene Surfaces

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Edward Steers, Jr. Research Biologist LCB, NIADDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Protein Chemistry

INSTITUTE AND LOCATION
NIADDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01AM25,042-03 LCB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Sickle Cell Disease: The Rheological Effects of Intracellular Polymerization

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	Clemenceau Acquaye	Visiting Fellow	LCB NIADDK
Others:	Alan N. Schechter	Chief	LCB NIADDK

COOPERATING UNITS (if any)
BEIB (E.L. Walker)

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Macromolecular Biology

INSTITUTE AND LOCATION
NIADDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
1.1	1.1	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The pathophysiology of sickle cell disease at the cellular and tissue level is poorly understood despite detailed information about the physical chemistry of hemoglobin S solutions. During the last few years we have been studying the intracellular polymerization of hemoglobin S and have detected polymer at high oxygen saturation. The present program is a multifaceted approach to study the rheological effects of polymer formation in red cells using electron microscopy, cell aggregation, cell filterability and cell viscosity measurements.

We are using electron microscopy to study the amount and physical properties of the polymer of deoxyhemoglobin S that forms in sickle erythrocytes at various oxygen saturation values. The electron microscopic studies are being correlated with results from nuclear magnetic resonance spectroscopy and other methods to examine the physical, physiological and clinical variables that affect intracellular polymerization of deoxyhemoglobin S. These studies will allow us to test various hypothesis about the pathophysiology of sickle cell disease, specifically the relationship of the amount and length of the polymer to changes in cell rheology. These direct studies of polymer have been correlated with aggregation, filterability, and viscosity measurements in an attempt to understand how the amount of polymer and the rate of its formation impedes the flow of blood in the microcirculation of sickle cell patients. To facilitate these studies we have worked with BEIB to develop a filtration apparatus which allows us to measure deformability properties at various oxygen saturation values. This apparatus has allowed us to measure precisely several of the variables that determine the flow properties of sickle erythrocytes. We will also try to correlate disease severity with these rheological measurements. In addition, we are studying several possible inhibitors of polymerization, especially analogs of diphosphoglycerate, in a continuing attempt to develop a pharmacological treatment of sickle cell disease and related symptoms.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01AM25,043-02 LCB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Induction of Differentiation in K562 Human Leukemia Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Yongji Wu	Visiting Fellow	LCB, NIADDK
Others:	Ann Dean	Research Chemist	LCB, NIADDK
	Alan N. Schechter	Chief	LCB, NIADDK

COOPERATING UNITS *(if any)*
Biochem. and Biophys. Division, FDA (Dr. W. Egan)

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Macromolecular Biology

INSTITUTE AND LOCATION
NIADDK, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.5	0.5	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have been studying the K562 human leukemia cell line in order to analyze the control of human erythroid differentiation and globin gene expression. We have shown that hemin causes induction of hemoglobin synthesis by increasing mRNA levels and that this process is fully reversible, without evidence of terminal differentiation.

We have studied the physiological properties of the embryonic and fetal hemoglobins synthesized in these cells as a response to hemin. We find that following induction of hemoglobin synthesis K562 cells demonstrate oxygen dissociation properties similar to human embryonic red cells. The P50 value of the hemoglobin is 20 ± 0.9 mmHg. The Hill coefficient is 2.5. Intracellular pH of K562 cells, measured by 31-P NMR, is 7.3. We also find that the levels of 2,3-diphosphoglycerate (DPG), a potent modifier of hemoglobin function, increase dramatically upon the induction of hemoglobin synthesis in K562 cells. In a typical experiment, DPG levels went from 8 nmoles/10^8 cells to 64 nmoles/10^8 cells upon induction of hemoglobin accumulation. For several experiments there was an approximately linear relationship between DPG and hemoglobin levels. The coordinate increase in DPG and hemoglobin levels has been demonstrated not only by an enzymatic method but also by 31-P NMR spectroscopy. These data suggest that DPG and hemoglobin levels are coordinately controlled in these cells. We are now investigating the molecular mechanism of the coordinate increase in DPG and hemoglobin levels in response to hemin induction.

In more recent experiments we have examined the effects of various analogs of 5-azacytidine which may also inhibit DNA methylation for their ability to induce hemoglobin synthesis in K562 cells. We find that 3-deazadenosine and several other analogs do cause induction. We are examining the molecular basis of this phenomenon as it may be relevant to the treatment of sickle cell anemia and thalassemia patients in an effort to increase fetal hemoglobin synthesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01AM25,044-02 LCB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on the Binding of Immunoglobulins to the Surface of Human Platelets

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Edward Steers, Jr.	Research Biologist	LCB, NIADDK

COOPERATING UNITS *(if any)*
N.R. Shulman, Clinical Hematology Branch, NIADDK

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Protein Chemistry

INSTITUTE AND LOCATION
NIADDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been transferred to the Clinical Hematology Branch, NIADDK

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01AM25,045-01 LCB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chromatin Structure and Transcriptional Activation in K562 Human Leukemia Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	Margaret A. Flanagan	Guest Worker (NRSA Fellow)	LCB, NIADDK
Others:	Ann Dean	Research Chemist	LCB, NIADDK
	C. Michael Fordis	Medical Staff Fellow	LCB, NIADDK
	Alan N. Schechter	Chief	LCB, NIADDK

COOPERATING UNITS *(if any)*
Medicine Branch, NCI (Dr. J. Whang-Peng and Ms. T. Knudsen)

LAB/BRANCH
Laboratory of Chemical Biology

SECTION
Section on Macromolecular Biology

INSTITUTE AND LOCATION
NIADDK, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The K562 cell is being studied as a model of human globin gene expression. K562 cells express embryonic and fetal hemoglobins but not hemoglobin A, β-globin chains, nor β-globin mRNA. Understanding the pattern of globin expression in K562 cells may yield insight into globin expression and hemoglobin switching in normal cells. Previous work on the integrity of the β-globin gene has been extended to show that chromosomal alterations do not account for the pattern of β-like globin expression seen in K562 cells. In addition, *in situ* hybridization has been used to assign the β-globin gene locus to the chromosome region 11p11.2 to 12.

Presently, studies are underway to correlate the observed pattern of sensitivity and hypersensitivity to DNase I digestion to possible alterations in chromosome structure in the transcriptionally active β-globin gene. This is being done by sedimentation analysis of chromatin fragments. Additionally, future experiments are planned to footprint non-histone proteins binding to the flanking regions of active globin genes. These proteins are putative *trans*-acting factors which may have a causal relationship to gene transcription.

ANNUAL REPORT
LABORATORY OF PHYSICAL BIOLOGY
NATIONAL INSTITUTE OF ARTHRITIS, DIABETES, AND DIGESTIVE AND KIDNEY DISEASES

Muscle Contraction

The idea that the actomyosin cross-bridge in muscle cells has more than a single configurational state and that contractile force is developed by a transition between two such states figures prominently in theories of muscle contraction. Results from several lines of study in our laboratory have provided direct evidence for this view and have defined conditions in which the different structural states can be studied.

Mechanical properties of relaxed muscle. At low ionic strength the stiffness of rabbit psoas muscle fibers in ATP solutions during rapid stretch is more than half the rigor value; during slow stretch the stiffness is close to the relaxed value. Thus rapidly reversible actomyosin cross-bridges appear to form in muscle fibers in the presence of ATP at low ionic strength. Similar experiments show that the attachment and detachment rates of myosin to actin in the presence of pyrophosphate (PPi) or adenyl-5-yl-imidodiphosphate (AMPPNP) are several orders of magnitude slower than in the ATP system.

When a muscle in PPi or AMPPNP solution is rapidly stretched, the actin and myosin filaments are displaced relative to each other and the attached cross-bridges develop force. The rate at which this force decays is a measure of how rapidly cross-bridges detach. In either PPi or AMPPNP solution, the cross-bridges detach with a wide range of rate constants which span as much as three decades. The number of cross-bridges attached to actin after force has decayed is roughly the same as before the force decays. Thus it appears that initially the cross-bridges are at equilibrium with most of the crossbridges bound to actin; the rapid stretch strains the cross-bridges so they detach from one actin and reattach to another in a less strained configuration. The rate-constant for detachment is determined by events at the active site (i.e. binding of nucleotide or analogue). (Schoenberg)

Equatorial X-ray diffraction of single muscle fibers. Single skinned rabbit psoas fibers were used to record equatorial X-ray diffraction patterns during isometric contraction with force levels ranging between 4 and 100% of full Ca^{++} activation. The intensity ratio of the first two reflections (I_{11}/I_{10}) increased almost linearly with force, similar to results obtained from the frog sartorius muscle, where the force was generated by rapid cooling contracture. The linear variation with force shows that the intensity ratio is a sensitive measure of the number of cross-bridges in the force-generating state. In addition to the intensity data, it was also found that the lattice spacing of the fiber decreased from 438Å with increasing force. Spacing appeared to level off at 380 Å when force reached approximately 80% of the maximum value, indicating that radial force generated by the crossbridges is a highly nonlinear function of the axial force. (Brenner, Yu)

To investigate the structural changes associated with cross-bridge formation in relaxed skinned rabbit psoas muscle fibers at low ionic strength (μ), higher order equatorial X-ray reflections were obtained beyond [1,1] from the relaxed and rigor states between μ = 20 to 100 mM. Axially projected two dimensional electron density maps were constructed based on intensities of five reflections ([1,0], [1,1], [2,0], [2,1] and [3,0]), with phase assignment (+,+,-,+,+). One of the main features of the density maps is that the backbone of the thick filament is resolved from the myosin heads which are mostly concentrated in a surrounding annular shell. As μ is lowered from 100 mM to 20 mM in the relaxed muscle, mass redistribution causes net loss in the region surrounding the thin filaments where the projected density originating from the myosin heads is less concentrated. The part of the annular shell where the projected density from myosin heads is highly concentrated shows less change. In contrast, in transition from relaxed to rigor state, the most severe net loss is in the region where the density from the myosin heads is highly concentrated. These patterns of mass redistribution provide evidence that the modes of cross-bridge attachment are different in the low ionic strength and the rigor state. (Yu, Brenner)

Two dimensional X-ray diffraction. Additional evidence that the configuration of the cross-bridge that forms in low μ relaxing solution is significantly different from that of the rigor bridge was obtained from the two-dimensional X-ray patterns of muscles in the various states. The distribution of intensity along the 59 Å actin-based layer line peaked closer to the meridian in rigor than in normal μ relaxing solution, presumably because the scattering units are larger in rigor due to decoration of actin by S-1. In low μ relaxing solution the intensity distribution along the layer line was close to that in normal μ, even though more than half the rigor number of cross-bridges were present. The result shows that S-1 binding to actin is more flexible in low μ than in rigor. (Matsuda, Podolsky)

The configuration of the cross-bridge in AMPPNP at normal μ is more "rigor like" than in ATP at low μ . In AMPPNP the myosin-based layer lines were markedly attenuated and the distribution of intensity along the 59 Å layer line peaked close to the meridian, as in the case of rigor. These structural observations are consistent with the mechanical properties of the AMPPNP bridge described above (Collett, Podolsky).

Measurements of sarcomere length in situ. The laser diffraction technique developed in this laboratory for measuring the sarcomere length of human skeletal muscles was used on cadavers and on several patients at the George Washington University Medical Center during forearm tendon transfer operations. The sarcomere length in cadavers with the hand in the "position of function" was found to be 2.4 μm. The use of clinical judgement during tendon transfer operations usually resulted in a sarcomere length greater than this value, as measured by the laser technique. A sarcomere length of 2.4 mμ could be quickly established using the laser device. (Podolsky, Brenner, Adams, Fleeter)

Cell Membrane

Self assembly of lipid bilayers. According to the principles governing equilibrium phase diagrams, an equilibrium state defined by temperature and chemical potential will form spontaneously when appropriate conditions exist. Thus, if lipids extracted from cells that are grown at a specific temperature can be shown to form the surface bilayer state at the same temperature at which the cells were grown, then the surface bilayer critical state must also exist in the cell membrane. This theory has been tested with *B. stearothermophilus* grown at 49° and 59°, and *E. coli* grown at 20° and 30°. Bacteria were used because they are known to modify their membrane lipids to adapt to each growth temperature. The lipids were extracted, aqueous dispersions of the lipids were prepared, and the surface pressure-temperature phase diagram was obtained for each membrane preparation. The bilayer critical temperatures that were observed for these lipids coincided with the growth temperatures of the organisms from which the lipids were extracted. Thus these cells assemble lipid bilayers in membranes under the same conditions that exist in the lipid dispersion. These results show that the critical state represented by surface bilayer formation also exists in these cell membranes.

To examine whether this relation also exists in mammalian cells, lipids were extracted from red blood cell membranes, and the surface pressure-temperature phase diagram for aqueous dispersions of these lipids was measured. The surface bilayer critical temperature occurred at 36 ± 1°, in good agreement with the normal body temperature of the cells. (Gershfeld)

Magnetic filtration of malaria infected erythrocytes. A simple, rapid technique for identifying red blood cells infected with malaria parasites would be useful clinically. To accomplish this, a magnetic filtration device was set up which has the ability to detect hemozoin, a paramagnetic metabolic product of hemoglobin found in malaria infected erythrocytes. The magnetic filter can concentrate infected erythrocytes by 30 to 100 times over the control. It is expected that the magnetic filter may be useful in reducing the number of false-negatives obtained in laboratory assays for malaria-infected cells using the light microscope. (Murayama, Garges)

Macromolecular Structure Determined by Radiation Inactivation of Biological Functions

The determination of molecular weights by radiation inactivation is of increasing interest and acceptance. Recently completed studies from this laboratory have included the β-adrenergic receptor from frog and turkey erythrocytes. The active unit for ligand binding was found to be a single polypeptide subunit, in excellent agreement with the molecular weight found by gel electrophoresis of purified receptor. Another project concerned is the glucocorticoid receptor from murine T-cell lines; the cytoplasmic and nuclear forms of the receptor both showed the same target size. This study is unique among radiation studies of receptors. Rather than using radiation to destroy the structures capable of binding the ligand, the radiation was used to release pre-bound hormone. It was necessary to devise this approach since the cytoplasmic form is a precursor to the one in the nucleus. The anticipated result considered the nuclear form to be a proteolyzed variant of the cytoplasmic form. Thus radiation inactivation of the larger precursor would severely complicate the study of binding to the smaller nuclear receptor. The conclusion from this unorthodox analysis is that the structures binding the glucocorticoid hormone in the cytoplasm are the same size as those found in the nucleus, which suggests that the transformation of cytoplasmic to nuclear forms involves neither proteolytic clips nor peptide addition.

Another major radiation study of lactose transport was completed. The lactose carrier protein in E. coli vesicles was irradiated and subsequently extracted and reconstituted into proteolipsomes. These reconstituted systems were then tested for transport activity. The target size observed was 45-50 kDa, in agreement with the known protein polypeptide subunit. However, if the E. coli vesicles were energized with electron donors before irradiation, the observed target size was doubled to 85-100 kDa, suggesting the functional requirement of a dimer. But even more striking was the target size observed in these energized vesicles when the proton electronchemical gradient was collapsed with m-chlorophenylhydrazone; the functional unit reverted to the monomeric size. These results indicate that the lactose carrier protein is in a dimeric state when energized, and the unenergized state is either monomeric or possibly dimeric but with very reduced interactions between the two polypeptides. (Kempner)

Insect Metamorphosis

The binding of blowfly ubiquitin to other proteins is much tighter than is the case in vertebrate tissues. Among several procedures tried to release such bound ubiquitin, only prolonged hydrolysis has been successful. The amount of bound ubiquitin is several fold higher than the titer of the free protein, but the profiles for both are similar during the insect life cycle. Pure blowfly ubiquitin has been isolated and characterized. It appears to be very similar, if not identical, to calf thymus and red cell ubiquitin. The ATP-ubiquitin proteolysis system from either flies or rabbit reticulocytes (where it is known to be highly active) do not hydrolyse the insect storage protein calliphorin. While crude lysosomal preparations from flies attack two unnatural proteins very rapidly, they hydrolyse the putative natural substrate (calliphorin) only very slowly. The pH optimum for this slow reaction is about 4, indicative of a lysosomal, catheptic mediated, hydrolysis. (Levenbook, Bauer)

Sense of Rhythm

The communication systems of fireflies are especially instructive because both sending and receiving make use of a signal modality almost unique among animals: flashes of visible light; because they are usually coded in readily quantitated parameters; and because much of the associated behavior can be referred directly to physiological events in the nervous system.

The major accomplishment of the year was the analysis of over two thousand measurements of photic latency of males of *P. concisus*. The latencies came from long series of light-emitting diode signals presented in rhythms ranging from signal per 1.5 s to one signal per 9 s. Series of consecutive (1:1) responses at frequencies of 1 per 1.5 s or 1 per 1.75 s included a considerable number of putative responses with latencies in the 300 to 600 ms range which often seemed to fall into discrete 250-350 and 550-700 ms classes. Above driving frequencies of 1 per 2 s negative phase responses predominated, indicating timing-cycle resetting as in Oriental fireflies, but with the difference that flashing period tended concurrently to lengthen. With a frequency of 1 per 9 s there were practically no responses earlier than 2 s post-signal and flashes thereafter were randomly timed. The overall conclusions are that the *P. consisus* pacemaker is susceptible to resetting, is excited by rhythmic stimulation *per se*, and exhibits two categories of reflex response much shorter than the reset (free run) cycle. (Buck, Hanson)

High Resolution Electron Microscopy

Paracrystals of human psoriatic keratin. When keratin subunits extracted from psoriatic scales are assembled under conditions which would normally yield epidermal keratin intermediate filaments (IF), large paracrystalline aggregates are found among the assembly products. Both to futher our knowledge of keratin structure and to investigate the psoriatic conditions, these paracrystals have been analyzed by electron microscopy and diffraction methods. The paracrystals are bundles of fibers close-packed with an average lateral spacing of 5.1 nm. Individual fibers have a prominent axial repeat of 5.5 nm and a less conspicuous repeat of 16.5 nm. Because their diameter is much less than that of IF, it is likely that these filaments are breakdown products or partially assembled structures, possibly at the "protofibrillar" level. A further indication in support of this hypothesis is given by the correlation of the 16.5 nm repeat with the length of a tract of coiled-coil α-helix deduced from primary sequence information. (Trachtenberg, Steven, Steinert)

Molecular composition of vesicular stomatitis virus (VSV). Although some aspects of VSV assembly have been elucidated in detail, considerable uncertainty still surrounds the question of the molecular composition of the virion. Current estimates of its content of the various structural proteins vary over a five-fold range. To clarify this question, STEM mass measurements were made of virions, pronase-shaved virions (the enzyme digests the G-protein protrusions), and purified nucleocapsids. The mass of the virion was 265 Mdaltons (Md), and it contained 1250 copies of N-protein, 1820 copies of M-protein, 1200 copies of G-protein, 50 copies of L-protein and 470 copies of NS-protein. The remaining mass is accounted for by a single molecule of ssRNA (3.8 Md) and 56 Md of lipids, giving mass ratios of protein : lipid : nucleic acid of 203 Md : 56 Md : 3.8 Md. In addition, the length of the helical nucleocapsid has been evaluated by four independent electron microscopic methods, two of which give values of 3.4 μm (inferred to represent a compact state of the nucleocapsid) and two of which give values of 4.0 μm (inferred to represent a distended state). (Steven, Thomas)

Assembly-dependent conformational changes of the gp23 surface lattice of bacteriophage T4D capsid. During the course of T4 head assembly, gp23, its major capsid protein, assumes a specifically regulated sequence of conformational states. Conformational changes between successive states are irreversible, and are also remarkable in that they are effected *in situ* on the polymerized protein, and involve large-scale structural changes (such as a 20 Å increase in the hexagonal lattice constant). By combining differential scanning calorimetry with high resolution electron microscopy, the following results have been obtained:
(i) The 45° endotherm of the uncleaved/unexpanded surface lattice probably repersents denaturation of the amino-terminal portion of gp23 (M_r 56,000) which is removed by proteolytic cleavage to yield gp23* ($M_r \sim 47,000$).
(ii) There is a range of thermodynamically distinct but structually indistinguishable states of the cleaved/expanded surface lattice, with melting temperatures of 73-81°. This variation depends on two factors: (1) the mutant used, 20 (amE481) producing polyheads that melt 4-5° lower than those of other mutants; and (2) the fraction of gp23 cleaved to gp23* before the expansion transformation takes place.
(iii) Incorporation of *hoc* protein into the expanded surface lattice has no substantial stabilizing effect on the surface lattice, but introduces a new endotherm at 68°. This event corresponds to denaturation of *hoc* (which thus becomes protease-sensitive), and results in a visible change in the part of the surface lattice structure attributed to the presence of *hoc*.
(vi) Incorporation of *soc* protein results in an increase in melting temperature by 5-6° and by a 50% increase in enthalpy of denaturation of gp 23*: *soc* does not exhibit an endotherm and does not appear to be thermally denaturable.
(Steven, Ross)

Cholesterol content of coated vesicle membrane. Earlier electron microscopic experiments using the cholesterol probe filipin led to the concept that membranes of coated pits and coated vesicles are specifically depleted in cholesterol and that this mechanism is responsible for sustaining the difference between the cholesterol content of plasma membranes and that of membranes of intracellular compartments. This ideal was tested by cytochemical electron microscopy with filipin, using highly purified preparation of liver coated vesicles (LCV) as well as LCV whose clathrin coats had been removed by dialysis at pH 8.0 (L-UCV). Biochemical determinations of the relative amounts of cholesterol, phospholipid, and protein show that both LCV and L-UCV contain cholesterol in amounts normal for plasma membranes, and that morphologically, L-UCV evince the normal cholesterol-positive response to filipin. The observation that LCV membranes are not distorted in the characteristic way by filipin was shown to be due to the stabilizing influence of the clathrin coat. In consequence, the attribution of a cholesterol-filtering property to coated pits and vesicles is no longer tenable. (Bisher, Steer, Steven)

Electrophoresis of coated vesicles. The broad size range (50-150 nm) of clathrin coated vesicles from liver cells may be indicative of more than a single origin region for these particles. Two peaks have been found in the electrophoretic migration of the vesicles in dilute agarose gels, and methods are being developed for eluting adequate amounts for their characterization. Gel electrophoresis data also indicate that the surface charge densities of brain and liver coated vesicles differ significantly. Since this is contrary to current models, in which coated vesicles from all cells are covered by similar clathrin structures, additional experiments in free solution are planned. (Gottlieb, Steven)

Mass distribution of clathrin cages assembled in vitro. The self-assembly properties of clathrin was studied by analysing the mass distributions of particles assembled *in vitro* out of clathrin triskelions. A wide range of particle masses was found under all assembly conditions. In addition to the cages of masses 23MD and 18MD, other types of cage with masses of 26 MD, 36 MD, and 42 MD were also found. On average, liver clathrin tends to form more massive particles, although this issue is complicated by the tendency for formation not only of cages that are empty, but also of cages that contain variable amounts of internalized material. On average, cages assembled at pH 6.05 are more massive than those assembled at pH 6.5, and the pH 6.05 populations also contain a higher incidence of larger particles. These appear, in most instances, to consist of smaller particles enveloped by an outer clathrin shell. (Steven, Steer, Bisher)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 27000-22 LPB

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Mechanism of Muscular Contraction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Richard J. Podolsky, Chief, LPB/NIADDK
Leepo C. Yu, Research Physicist, LPB/NIADDK
Bernhard Brenner, Visiting Associate, LPB/NIADDK
Takshi Matsuda, Visiting Scientist, LPB/NIADDK
Brian Collett, Staff Fellow, LPB/NIADDK

COOPERATING UNITS *(if any)*

Dr. John Adams, Dept. of Orthopedics, George Washington University School of Medicine.

LAB/BRANCH

Laboratory of Physical Biology

SECTION

Section on Cellular Physics

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.6	3.6	2

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Contractile force in muscle fibers is generated by cross-bridges that form between the myosin-containing and actin-containing filaments and hydrolyze ATP. During shortening, the two types of filaments slide relative to each other. The structural changes associated with cross-bridge formation have been measured by X-ray diffraction techniques, using radiation from both rotating anode generators and synchrotron sources. The diffraction patterns were recorded with both one-dimensional, electronic, position-sensitive, X-ray detectors and film. Muscle preparations were made from rabbits and frogs, and and they included single skinned fibers and small bundles of skinned fibers.

The diffraction pattern from the cross-bridge that forms in low ionic strength relaxing solution was found to be significantly different from that of the rigor (ATP-free) cross-bridge. This finding shows that actomyosin cross-bridges have two significantly different structures, and it suggests that contractile force in muscle fibers may be generated by a transition between these two actomyosin configurations.

The ordering of the myofilaments in relaxed rabbit muscle was found to depend on the electric charge on the filaments. Thus the double hexagonal arrangement of the myofilaments appears to be maintained by a balance between attractive and electrostatic repulsive forces.

A laser diffraction technique for adjusting sarcomere length of muscles during tendon transfer operations was used successfully in a clinical setting.

The overall goal of the project is to find out how the actomyosin system in muscle fibers produces force and motion, and how the mechanical responses are regulated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 27001-10 LPB

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Contractility and Excitability of Skeletal and Cardiac Muscle

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Mark Schoenberg, Medical Officer, LPB, NIADDK

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Laboratory of Physical Biology

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Section on Cellular Physics

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.1	1.1	0

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☐ (a) Human subjects ☐ (b) Human tissues XX (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have recently discovered that myosin crossbridges bind to actin in relaxed muscle and interact in a manner characterized by rapid, reversible attachment and detachment. We have also found that the rate of crossbridge attachment and detachment depends upon the nucleotide or nucleotide analogue bound to the active site. Since the attachment/detachment rates in the presence of ATP are so rapid as to make measurement difficult, we have recently concentrated on studying the behavior of actin-myosin crossbridges in the presence of pyrophosphate (PPi) or adenyl-5-yl imidodiphosphate (AMPPNP) where attachment/detachment rates are slower. We detect crossbridge detachment by measuring the decay of tension following rapid stretch of an isolated single muscle fiber which has had its outer membrane removed to facilitate equilibration with experimental solutions.

We have found that detachment of the crossbridges in 4, 1, or 0.25 mM PPi or AMPPNP does not proceed with a single rate-constant. Rather, it proceeds over a wide range of rate-constants with the ratio of the fastest and slowest rate-constants as great as 10^2-10^3. Since the number of crossbridges attached at the end of the force decay is the same as at the start, the simplest interpretation of the force decay is that strained cross-bridges are detaching from one actin and reattaching to another actin in a position of lesser strain. The reason this process proceeds over such a wide range of rate-constants is under intense investigation; it may be related to competition between adjacent myosins for available actin sites. If so, force maintenance in a muscle fiber may be highly cooperative when large numbers of crossbridges are attached. This may have an important role in the regulation of skeletal muscle.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM-27002-21 LPB

PERIOD COVERED October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Sense of Rhythm

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

John B. Buck Research Physiologist, Laboratory of Physical Biology, NIADDK

COOPERATING UNITS *(if any)* Dr. Frank E. Hanson, Dept. of Biology, Univ. of Maryland, Catonsville, Md.; Dr. James F. Case, Dept. Biological Sciences, Univ. of California, Santa Barbara, California.

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SECTION

Comparative Physiology Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2	1	1

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The communication systems of fireflies are especially instructive because both the sending and the receiving use a signal modality almost unique among animals: flashes of visible light; because they are usually coded in readily quantitated parameters; and because much of the associated behavior can be referred directly to physiological events in the nervous system.

During several years we studied flash control in species of Oriental firefly that have the peculiarity of flashing synchronously in large congregations and in which the synchronization occurs because the photic response has a latency approximating the free run flashing cycle i.e., stimulation at any phase of the cycle sets the pacemaker back to zero time. In recent years we have investigated two American species of firefly which synchronize on a much more limited scale and in which the entrainment mechanism has seemed to depend on direct reflex evocation of premature flashing (i.e., response with a latency much shorter than the free run period). Analysis of video field recordings of photic triggering in the species Photinus pyralis showed that premature flashing (350 ms latency) is only evoked by photic signals in the latter half of the flashing cycle (from about 2.8 to 5.6s). However, in P. concisus, a closely related species in which the free run cycle is 2s rather than 6s, we find that males trigger males at a latency of 600 ms, the same as the female's normal response latency, and apparently cannot distinguish real from simulated female answers. This species thus presents a major behavioral puzzle.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM-27003-15 LPB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The dynamic properties of cell membranes and related systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Norman L. Gershfeld, Research Chemist, Laboratory of Physical Biology, NIADDK

COOPERATING UNITS *(if any)*

None

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SECTION
Comparative Physiology Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2	1	1

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The dynamic properties of cell membranes is the principal focus of this project. The most recent studies have examined spontaneous mechanisms of lipid bilayer assembly in a system that consists of synthetic and naturally occurring phospholipids dispersed in water. The lipid bilayer is generally accepted as the principle lipid structure in cell membranes. Equilibrium techniques have been developed that measure the chemical potentials of the lipids as a function of temperature. In principle, these phase diagrams provide a general framework for describing all lipid states that form spontaneously. This approach has led to the finding of the spontaneous formation of a single lipid bilayer state that exists at a singularity in temperature. This state has been shown to exist at a critical temperature (BCT). The BCT has been described with respect to the chemical structure of the phospholipid (polar and hydrocarbon chain moieties) and to the dependence of the BCT on mixtures of phospholipids.

As a test of the relevance of the BCT to the assembly of lipid bilayers in cell membranes, bacteria were grown at different temperatures; the membrane lipids were extracted, and dispersed in water; and the BCT for each of these lipid mixtures was obtained. For all cell systems that were studied the BCT was identical with the temperature of cell growth within an experimental error of ± 1.0°. Thus B. stearothermophilus grown at 49° and 59°, and E. coli grown at 20° and 30° exhibited BCT's at these same temperatures. Since the BCT is defined only by the temperature and composition of the membrane lipid extract, this constitutes a thermodynamic proof that the phase relations of the lipids in the cell membranes are those of the bilayer critical state. In principle, therefore, these cell membranes utilize the same process for the assembly of lipid bilayers.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM-27004-14 LPB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Physical chemistry of cell membranes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Melvin H. Gottlieb Research Chemist, Laboratory of Physical Biology, NIADDK

COOPERATING UNITS *(if any)*

Dr. C. J. Steer, Laboratory of Biochemistry and Metabolism, NIADDK
Dr. A. Crambach, Laboratory of Theoretical and Physical Biology, NIADDK

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Transport of a number of macromolecules from outside the cell to intracellular organelles is mediated by the incorporation of the macromolecules into clathrin coated vesicles. Coated vesicles from liver have an unusually broad size distribution with diameters ranging from 50 to 150 nm. Since the broad distribution may be indicative of the presence of more than one type of vesicle, the preparative size fractionation of liver coated vesicles has been undertaken. The method most extensively examined thus far is electrophoresis in dilute agarose gels, a technique recently described by other workers for studying viruses of the same size range as the coated vesicles. Experimental conditions have been found in which the liver coated vesicles, and also coated vesicles from brain, enter the gel and migrate within it in a controlled manner. The diameters of both types of vesicles computed from the dependence of migration velocity on gel concentration (using virus data for calibration) agree well with electron microscope measurements. Encouragingly, in a particular range of agarose concentrations two migration peaks occur in the case of the liver vesicles. Methods of scaling up the electrophoresis procedure, and eluting vesicles from the gel in high yield are currently being developed.

Extrapolation of the electrophoretic velocity data to zero gel agarose content indicates that the electrical charge density at the surface of the brain vesicle is about 30% greater than that of the liver vesicle. Since this is in conflict with current concepts, according to which coated vesicles from all cells should have similar surface properties, i.e. those of the clathrin coat, it is planned to measure the free solution mobilities of the two types of vesicles directly; this will be done by a Doppler shift technique in collaboration with a group at Syracuse University.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM-27006-27 LPB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Developmental biochemistry of insect metamorphosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Leo Levenbook Research Chemist, Laboratory of Physical Biology, NIADDK

COOPERATING UNITS *(if any)*
Dr. E. C. Weinbach, Laboratory of Parasitic Diseases, NIAID

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

(1) Our radioimmunoassay for ubiquitin -- a small, highly conserved "signal" molecule involved in ATP-dependent intracellular proteolysis -- recognizes only free ubiquitin. To measure total ubiquitin (protein bound + free) requires prolonged tissue hydrolysis; its profile is similar to that previously described for the free component i.e. highest titers in egg and adults, and lowest in larvae and pupae. Pure blowfly ubiquitin has been isolated and partially characterized. The ATP-ubiquitin proteolytic system from either the blowfly or from rabbit reticulocytes does not hydrolyse the blowfly storage protein calliphorin.

(2) Enriched lysosomal preparations from late pharate adult flies very actively hydrolyse bovine serum albumin and denatured hemoglobin; but they attack calliphorin at only 1-2% of the rate for the unnatural substrates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM-27008-25 LPB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biophysical studies of metabolic activity and control

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Ellis S. Kempner Physicist, Laboratory of Physical Biology, NIADDK

COOPERATING UNITS *(if any)*

Drs. H. Kaback (Roche Institute); S. Bourgeois (Salk Institute); H. Haigler (University California, Irvine); J. Harmon (American Red Cross); T. Nielsen (Baylor University)

LAB/BRANCH

Laboratory of Physical Biology

SECTION

Comparative Physiology Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Molecular weight of specific biopolymers is an important parameter in characterizing the molecule, its position and orientation in situ and its interactions with other compounds. Measurements of molecular weight are accomplished by several different techniques which depend on chemical or physical properties of these structures, and all of which demand purified samples. An alternative method is that of radiation inactivation. By means of target analysis it is possible to evaluate a molecular weight which depends on different properties, the functional activities of the specific biopolymer. It is an important feature of this technique that crude samples can be used.

The physical basis for this approach has been described long ago, but as the method is applied to more complicated systems and more complex structures, new questions arise. These involve interactions between subunits of proteins, effects of a lipid environment as in membranes, or the presence of carbohydrates. Experimental procedures which modify the natural order of things -- treatment with detergents, for example -- could also change the radiation-sensitive unit detected by enzymatic activity or receptor function. Analysis of these effects is an important phase of this project on molecular weight determination by radiation inactivation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 27009-06 LPB

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Macromolecular Structure

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Alasdair C. Steven, Visiting Scientist, LPB, NIADDK

COOPERATING UNITS (if any)

Other collaborators on specific projects are identified below, in the detailed account of these activities (cf. project description).

LAB/BRANCH

Laboratory of Physical Biology

SECTION

Section of Cellular Physics

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues XX (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Virus structures have been analyzed in the cases of (i) Tobacco mosaic virus, where the average radial density distributions of both the virion and RNA-free polymers of TMV coat protein have been derived from dark-field electron images of unstained specimens. Both particles have outer diameters of 17.4nm, central holes 3.5nm in diameter, and the virion contains an additional density peak at a radius of 4nm contributed by the packaged RNA molecule. (ii) Vesicular Stomatitis Virus has also been analyzed by this technique. Unstained, its full diameter is about 84nm, and distinct radial features have been identified with the external glycoprotein spikes, the membrane envelope, and the coiled nucleocapsid containing the RNA located at a radius of 16nm. (iii) Conformational transformations of the surface lattice of Bacteriophage T4 capsid have been characterized by combining differential scanning calorimetry with electron microscopy. In all states studied, the inter- and intrasubunit bonds are found to be comparably stable in respect of thermal perturbation, as the polyheads denature as single cooperative units at temperatures characteristic for each of the different conformational states. Two minor endotherms were also observed which are inferred to represent, in one case, denaturation of the N-terminal portions of the gp23 molecules, and in the second case, denaturation in situ of hoc proteins in the mature capsid structure.

In addition, we have studied the membranes of coated vesicles purified from rat liver and found them to contain cholesterol in amounts typical for hepatocyte plasma membranes, but to be morphologically susceptible to filipin, a cytochemical probe for cholesterol, only when their clathrin coats are removed. In consequence, the attribution of a cholesterol-filtering capacity to endocytic coated pits is no longer tenable. Finally, we refined our general structural model for Intermediate Filaments, and initiated a project to determine the helical organization of bacterial pili.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM-27011-02 LPB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Magnetic "filter" for Concentrating Malaria Infected Human Erythrocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator)* (Name, title, laboratory, and institute affiliation)

Makio Murayama, Research Chemist, Biological, LPB, NIADDK

COOPERATING UNITS *(if any)*

Lawrence M. Garges, Commander, Immunologist, U.S. Naval Res. Institute

LAB/BRANCH

Laboratory of Physical Biology

SECTION

Section on Cellular Physics

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

SUMMARY OF WORK: Super magnets which are made from rare earth-cobalt sintered powder magnets are being used in pairs with a Pasteur pipet "filter" inserted in between them. The "filter" consists of 14 cm. Pasteur pipette packed with 25 micron diameter 430 grade stainless steel wire. The underlying theory of the "filter" is as follows: when P. falciparum enters a red blood cell, it metabolizes the hemoglobin into a paramagnetic pigment, hemozoin. Hemozoin consists of aggregate of protoporphorin IX. Paramagnetic particles are attracted to very fine stainless steel wire because it provides the maximum magnetic field gradient i.e., dH/dZ = max. We have been able to demonstrate that the super magnetic "filter" can concentrate the infected erythrocytes by 30 to 100 times over the control. The magnetic filter is useful in minimizing the number of false-negative laboratory diagnosis i.e. when the number of malaria infected red cell is low, it is not readily found under the microscope and the patient will be diagnosed as not having any infected cells. It could be used also to separate the young P. falciparum from the old in order to start a synchronous culture of the parasite; since the young P. falciparum will have produced but a small amount of hemozin, therefore these cells will not be trapped in the "filter".

ANNUAL REPORT OF THE LABORATORY OF CHEMICAL PHYSICS
NATIONAL INSTITUTE OF ARTHRITIS, DIABETES, AND DIGESTIVE AND KIDNEY DISEASES

Research in the Laboratory of Chemical Physics is mainly devoted to applying spectroscopic methods to the study of molecular and cellular structure and dynamics. Spectroscopic techniques include nuclear and electron magnetic resonance, infrared and Raman spectroscopies and many specialized techniques, including nanosecond absorption spectroscopy, electric-field-induced linear dichroism, X-ray spectroscopy, and multiphoton ionization spectroscopy. Systems currently under investigation include proteins, nucleic acids, natural and artificial cell membranes, red blood cells, retinal photoreceptors, and a variety of small biological molecules.

Nuclear magnetic resonance (NMR) continues to provide a valuable tool for examining molecular structure, conformation and motion. Bax has initiated a research program to develop advanced new NMR pulse methods and to apply them to elucidation of structure in a variety of biochemically important molecules. For example, he has devised a powerful technique that allows long-range connectivity between nuclei (2-3 bonds) in an organic compound to be determined very efficiently. With his co-workers he has applied the technique to the assignment of carbonyl resonances in peptides and the elucidation of interglycosidic linkages in carbohydrates. The capsular polysaccharide from E. coli capsule type 53 as well as a number of benzanthracene and phenanthrene derivatives have been studied in this new way. In other NMR studies, Becker a co-workers have elucidated relaxation mechanisms in ^{57}Fe (a nucleus of very low NMR sensitivity) in ferrocene, porphyrin and bipyridyl derivatives. They have also analyzed the relaxation of ^{13}C in tertiary butanol as it is affected by hydrogen bonding.

Ziffer and Ito have extended their studies on enantioselective hydrolysis using Rhizopus nigricans by examining the absolute stereochemistry of the alcohols formed from a series of medium-ring 2-cycloalkenyl acetates. These hydrolyses were shown to yield chiral alcohols of high optical purity whose absolute stereochemistries were established as R by chemical transformations to compounds of known absolute stereochemistry. In addition, the chiral alcohols were used to determine the applicability of a rule recently proposed by Harada and Nakanishi, which relates the signs of the circular dichroism bands of p-substituted benzoates of allylic alcohols to their absolute stereochemistry, to esters which may exist in many more conformations than are available to the corresponding five-, six- and seven-membered analogues. The Harada-Nakanishi rule was successful in relating the experimentally observed circular dichroism bands to the calculated conformations of the esters.

Levin and coworkers have continued their applications of Raman and infrared spectroscopy in probing the dynamical, conformational and packing properties of both model and intact membrane assemblies. Clathrin, the major structural protein associated with both coated pits and coated vesicles, has been implicated in the dynamics of various endocytic processes. In an attempt to define the mechanisms involved in the transition from uncoated membranes to clathrin coated pits and then to coated vesicles, Steer, Vincent and Levin investigated by infrared spectroscopy the lipid perturbations arising from the interactions of the clathrin coat with the bilayers of intact membrane assemblies. A temperature study of the lipid acyl chain stretching modes of isolated clathrin coated vesicles, uncoated vesicles and synaptic membranes indicates that the presence of the clathrin coat clearly introduces a bilayer perturbation leading to an increase in the average number of gauche conformers along the lipid chains. Since the lipid environments of the coated pit and coated vesicle are not significantly different from uncoated domains, the bilayer disorder induced by the clathrin coat may be a major factor responsible for membrane invagination and coated vesicle formation.

O'Leary, Ross and Levin examined the effects of anesthetic and non-anesthetic steroids on dipalmitoylphosphatidylcholine vesicles using high sensitivity scanning calorimetry and Raman spectroscopy. Although there was no correlation between anesthetic potency and effects of the steroids on the main gel to liquid crystalline phase transition, a significant correlation was observed between anesthetic potency and the magnitudes of the steroid induced transition broadening and lowering of the pretransition temperature. The bilayer perturbation results from insertion of the steroid into the acyl chain region of the membrane, rather than into the headgroup or interface regions of the bilayer. The efficacy of a steroid in perturbing the subtle structural and dynamical properties of the bilayer pretransition is dependent on the projection and bulk of substituents at the steroid C(17) position.

In an effort to understand the mechanisms in which solvents, such as water, influence the structure and behavior of biological membranes, O'Leary and Levin characterized by Raman spectroscopy the properties of dipalmitoylphosphatidylcholine (DPPC) dispersed in glycerol and in N-ethyl ammonium nitrate (EAN), a fused salt. In glycerol, as distinct from water, the acyl chains of DPPC form interdigitated, hexagonally packed gel phase lipid bilayers. For the EAN dispersions, attenuation of dipolar repulsive forces between neighboring headgroups by the solvent results in an ordered, orthorhombic subcell for the acyl chains. On melting of the low temperature lamellar state, the decreased free energy of transfer of the acyl chains from the lipid hydrocarbon region to the EAN solvent, in contrast to water, induces micellization, rather than liquid crystalline bilayer formation.

Although the pretransition and main gel to liquid crystalline phase transition in phospholipid bilayers have been the subject of extensive investigations, it is now clear that the lamellar "gel" phase is an intermediate metastable state. In order to understand more completely the properties of the stable "crystalline" state assumed by hydrated bilayers, Levin and Huang investigated the Raman and ^{31}P NMR spectroscopic properties and phase transition behavior of a highly asymmetric lipid molecule 1-stearoyl-2-acetoyl-sn-glycero-3-phosphocholine, dispersed in water. The order/disorder transitions of the hydrated system follow the scheme of interdigitated crystalline → interdigitated gel phase → micellar phase. Formation of the low temperature interdigitated crystalline phase required prolonged incubation times at 0°C. Raman spectroscopic evidence leads to the conclusion that the long sn-1 chains of the asymmetric lipid span the hydrocarbon width of the lamella and therefore form interdigitated bilayers.

In determining the effect of headgroup constraints on membrane bilayer behavior, Kirchhoff and Levin examined the vibrational spectra of a series of multilamellar dispersions of cyclopentanoid analogues of dipalmitoylphosphatidylcholines. Since the analogues of DPPC exhibit an additional rigidity about the glycerol backbone, significant differences in packing, conformational properties and thermal behavior were observed between the liposomal preparations of the four members of the series and between bilayers comprised of unconstrained DPPC. A two-state model was used both to describe quantitatively the temperature dependence of the Raman spectra and to obtain a measure of the distribution of domain sizes.

Harris and Levin have examined the thermotropic behavior of a series of fully hydrated multilamellar dispersions of n-alkylphosphocholines by Raman spectroscopy. These single chain, ether linked systems undergo sharp lamellar to micellar phase transitions. The C_{15}-, C_{17}- and C_{19}- and the C_{14}-, C_{16}-, C_{18}- and C_{20}-chain systems form even and odd series with respect to their phase transition behavior. Polymorphic forms, whose phase transitions were 3 to 12°C below the stable structures, and their subcell packing characteristics were identified for all systems. (The C_{14}- and C_{20}-chain species, however, exhibit only single lamellar structures.)

Adams and Levin, using diode array Raman detection techniques, examined the sensitivity of the integrated Raman intensities of the C-H stretching mode region to bilayer reorganizations of model liposomes. This intensity probe is particularly sensitive to bilayer expansion effects, as, for example, the structural changes involved in the membrane pretransition.

Charney and co-workers have continued their study of large polymeric molecules oriented in pulsed electric fields. Using the great sensitivity of rotational diffusion coefficients to the length of polymer molecules, DNA sequence-dependent conformations and transitions have been investigated. Theoretical treatment of polyelectrolyte orientation in electric fields has been confirmed, while it has been found that experimental studies purporting to show that the nucleic acid bases of B-DNA are highly tilted away from the helical axis are probably incorrect. The B structure of DNA is more likely to be essentially that already found by X-ray fiber diffraction. Using electrodichroism it has also been found that the synthetic polynucleotide, poly(dA-dT) normally exists in the B structure and is twice as flexible as random sequence DNA. Values of the nucleation parameters and energy required to convert B form to Z form DNA have been obtained (Szu, Charney). A series of synthetic small peptides with differing conformations determined by circular dichroism have been studied for their antigenic properties. Their secondary structure in solution seem to be largely irrelevant to their antigenicity, which seems to depend on other molecular properties (Appella, Hansburg, Sussman, Szu, and Charney). Intermolecular forces between DNA double helices have been investigated both theoretically and experimentally. The observed force is independent of ionic strength but depends on the ionic species that coats the DNA surface. When two helices are pushed closer together than 0.8 to 1.2 nm, strongly attractive hydration forces are revealed (Rau, Parsegian).

Eaton and Hofrichter and their colleagues have continued to use laser spectroscopy to follow the molecular dynamics of hemoglobin as it binds to and releases ligands and to analyze their experimental findings by studying macromolecular models generated by computer-based simulations of molecular configuration and motion. Structural changes in hemoglobin subunits that occur when carbon monoxide is dissociated from one or more heme groups by an intense laser flash of nanosecond duration were studied. Using specially prepared tetramers in which either the alpha or beta subunits had their normal iron atoms replaced by non-CO-binding cobalt, it was possible to follow conformational changes induced in cobalt-containing subunits by photodissociation of the Fe-CO complexes in adjacent subunits. These changes precede the R-T conformational changes responsible for allosteric binding of oxygen by hemoglobin, and take place in 1-2 microseconds. They apparently reveal by direct experiment the communication of a structural change to one protein subunit by means of a change in the structure of a second subunit in contact with it - a mechanism thought to be important in regulation of activity in many biologically important molecules [Hofrichter, Eaton, Yonetani, Henry, Deutsch]. Studies of the details of conformational changes in heme proteins during and after photodissociation of ligands of differing sizes have been extended using myoglobin and a variety of bulky alkyl isocyanides as well as oxygen and CO. From study of the absorption spectrum of myoglobin as it rebinds a photodissociated ligand it was concluded that at least two structural relaxations with time constants of 30 nanosec and 1 microsec are present [Sommer, Henry, Hofrichter]. Computer simulations of the structural changes in heme after photodissociation have been carried out in order to assign the 1 microsec and 50 nanosec transient spectral changes to particular structural alterations in hemoglobin. So far, the computations suggest that changes in conformation in the heme itself are not responsible for the two relaxations [Henry].

The kinetic mechanism of polymerization of sickle cell hemoglobin has been investigated in more detail. A model involving both homogeneous and heterogeneous nucleation has been analyzed and shown to account quantitatively for all of the major kinetic features of hemoglobin S polymerization both in solutions and in red blood cells [Hofrichter, Eaton].

Szabo and collaborators have developed several theoretica advances that promote the analysis of relaxational processes in macromolecules and diffusion in solutions containing traps like those known to exist in cells. The time course of emission of fluorescence by electronically excited chromophores, such as the tyrosine and tryptophan residues in a protein or a dye molecule intercalated into DNA or a biological membrane, is known to depend on many physical factors. Among these are rotational diffusion of the excited molecule, heterogeneity of its environment and energy transfer to other electronic states in the same or nearby molecules. Since time-resolved measurement of fluorescnce emission anisotropy is a sensitive and frequently used measure of the reorientational dynamics of fluorophores in macromolecular systems, a comprehensive theoretical treatment of the time course of emission anisotropy as affected by molecular rotation and intermolecular energy transfer has been devised. This will provide a rational physical theory with which to interpret the many experimental studies of fluorescence emission anisotropy now being published by many laboratories and being analyzed only by empirical curve-fitting [Szabo].

Diffusion of ions and molecules in cells and subcellular organelles is the fundamental means by which nutrients, metabolites, and messengers move from place to place. Ordinarily diffusion is understood to proceed as if in a homogeneous solution even in cells of complex geometry containing many types of binding sites and obstructions. The usual methods of simulating the diffusion process by classical mathematical analysis or simple finite difference methods are often too inefficient computationally or break do entirely in such cases. New methods for treating diffusion in su complicated cases with improved algorithms and Monte Carlo methods have been devised [Lamm, Szabo, Shoup, Tsuchiya].

McDiarmid and colleagues have continued their investigations of the energy levels and transitions in conjugated polyenes such as those in vitamins A and D. Using excitation of the electronic systems by electron impact or by multiple photon absorption it has been possible to study energy levels in the molecules inaccessible to ordinary absorption spectroscopy. Spectroscopic differences between butadiene and 2,3 dimethylbutadiene have been observed with these methods and the second valence transition of the dimethyl derivative has been shown to be a thermally enhanced forbidden transition not seen in butadiene itself. Trienes, which are analogs of vitamin D in their spectra, have been found to have no electronically forbidden transitions in either the cis or trans isomers [McDiarmid, Sabljic, Doering].

Studies of applications of organic chemistry to biologically important problems have continued. Weiss has continued his collaborative efforts to improve the simplicity and efficiency of synthesis of di and polycyclic systems composed of cyclopentane rings and to understand the mechanisms of the synthetic reactions more fully [Weiss, Cook, Bhatnagar, Merlini]. Computerized methods of drug design have been used to explore improved inhibitors of the enzyme, aldose reductase, thought to be important in producing some of the severe secondary consequences of diabetes mellitus [Sharpless, Jennings, Adams, Kador].

Kon and his colleagues have continued their studies of orientation and deformation of erythrocytes by detecting the flow-induced orientation of paramagnetic lipid-soluble stable free radicals ("spin labels") dissolved in the cell membranes. Conditions under which the cells are so crowded together that they interact have been explored. The average deformation or orientation of the cells at a fixed flow rate shows two-phase dependence on hematocrit. In the rising phase, cell-cell interaction is not seen, while the declining phase at high hematocrits represents the interference between the cells. Cells having a reduced deformability produced by chemical cross-linking have been compared in their flow behavior with normal cells. Cross-linked cells interact with each other in a way that is less concentration-dependant than normal cells. Mixed populations of hardened and normal cells have also been studied [Kon, K.Kon, Takahashi, O'Bryan].

Studies of the cellular mechanism of excitation of retinal photoreceptor cells have continued. The possible role of cyclic nucleotide hydrolysis in releasing intracellular Ca++ by first acidifying the cytoplasm of the cells through production of free phosphate esters from cyclic ones has been explored. Cytoplasmic pH changes were produced by applying or removing permeant lipid-soluble weak acids or bases from the fluid bathing retinal rods. Acidification of the cells suppressed the physiological dark current much as light does. The effect of acidification could be suppressed by the permeant buffer, imidazole. However the action of light on the dark current was unaffected by imidazole. Related experiments in which the internal pH of the rods was monitored with fluorescent indicator dyes suggest the same conclusion [Yoshikami, Hagins].

A comprehensive method for quickly freezing living retinas under various conditions of light or dark and analyzing the rod cells for structural and soluble elements from Na through Cs by X-ray microanalysis in a scanning electron microscope has been developed. Permeabilities of the cells to Na, K, Rb, and Cl have been measured and a study of the intracellular Ca distribution is in progress [Foster, Yoshikami, Hagins].

The central laboratory computer of the Laboratory of Chemical Physics has been expanded by incorporating new high speed disk drives and improved peripheral data-collecting computers. The number of users has increased. Word-processing computers have been installed in the Laboratory office and staff training has begun. An Apollo molecular graphics terminal has been installed to allow molecular dynamics calculations and graphics as a routine part of the ongoing research of several groups [Jennings, Henry, Powell].

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AM-29000-12-LCP

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Nuclear magnetic resonance: new methods and molecular structure determination

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI :	Edwin D. Becker	Research Chemist	LCP, NIADDK
OTHERS:	Ad Bax	Visiting Associate	LCP, NIADDK
	Rolf Tschudin	Electronics Engineer	LCP, NIADDK
	Susanta Sarkar	Visiting Associate	LCP, NIADDK

COOPERATING UNITS (if any)
J. A. Ferretti, NHLBI; W. Egan, NCDB/FDA; J. S. Cohen, NCI; L. Sweeting, Towson State University; G. E. Maciel, Colorado State University; B. Hawkins, Colorado State University

LAB/BRANCH
Laboratory of Chemical Physics

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Section on Nuclear Magnetic Resonance

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.3	0.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A new NMR technique has been developed that facilitates structure determination and spectral assignment. The method has been used for complete structure determination and spectral assignment of the capsular polysaccharide from E. coli, capsule type 53. Complete ^{1}H and ^{13}C spectral assignment of a large number of polycyclic aromatic compounds has also been obtained with the new method.

Improvements for a number of other one- and two-dimensional experiments have been developed. A modification of the solid-state cross-polarization NMR experiment facilitates the application of this technique to nuclei with a very low magnetogyric ratio. A simple modification of the two-dimensional (2D) heteronuclear shift correlation experiment enables spectra to be recorded in the pure absorption mode, improving both sensitivity and resolution. A new one-dimensional technique has been developed that is superior in sensitivity to 2D experiments if only a small number of heteronuclear correlations have to be investigated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AM-29001-12-LCP

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular dynamics and vibrational characteristics of membrane assemblies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI :	Ira W. Levin	Research Chemist	LCP, NIADDK
OTHERS:	Timothy J. O'Leary	Medical Staff Fellow	LCP, NIADDK
	James S. Vincent	Guest Worker	LCP, NIADDK
	William H. Kirchhoff	Guest Worker	LCP, NIADDK
	William C. Harris	Guest Worker	LCP, NIADDK

COOPERATING UNITS *(if any)*
R. Adams, LCP-NIADDK; Clifford J. Steer, LBM-NIADDK; C. Huang, School of Medicine, University of Virginia; G. deHaas, University of Utrecht; and A. Hancock, University of Missouri

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Laboratory of Chemical Physics

SECTION
Section on Molecular Biophysics

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Vibrational Raman and infrared spectroscopy are used to probe the dynamical, conformational and packing properties associated with lipid-lipid and lipid-protein interactions in membrane assemblies. For example, clathrin, the major structural protein associated with membrane coated pits and cellular coated vesicles, has been implicated in the dynamics of various endocytic processes. Infrared spectrocopy was used to assess the lipid perturbations originating from the interactions of the clathrin coat with the bilayers of the intact, vesicle membranes. Spectra of the $\sim$2850 cm^{-1} symmetric methylene stretching modes of coated vesicles, uncoated vesicles and synaptic membranes were compared for a wide range of temperatures. Although the bilayer chains are intrinsically disordered because of their heterogeneous composition, further disorder in terms of the introduction of gauche conformers occurs on increasing the temperature of the system. In addition to each membrane system displaying a temperature dependence for the spectroscopic marker reflecting intrachain disorder, the clathrin coated vesicle systems manifest a greater lipid chain disorder, compared to the uncoated vesicles and synaptic membranes, at each temperature studied. This bilayer disorder induced by the clathrin coat may prove to be one of the major factors responsible for membrane invagination and coated vesicle formation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-AM-29002-11-LCP

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Chemistry of natural compounds, and synthetic organic chemistry

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Ulrich Weiss Research Chemist (Scientist Emeritus) LCP, NIADDK

COOPERATING UNITS *(if any)*
James M. Cook, Dept. of Chem., University of Wisconsin-Milwaukee
K. M. Madyastha, Dept. of Organic Chem., Indian Institute of Science, Bangalore
Surendra P. Bhatnagar and B. B. Singh, Reckitt & Colman of India, Bangalore
Lucio Merlini, Istituto di Biochimica Generale, Universita, Milano, Italy

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1	1	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

In continued cooperation with Dr. Cook and his group, the synthesis of di- and polycyclic ring systems composed of cyclopentane rings has been developed further, with particular emphasis upon development of synthetic approaches to biologically active compounds of this type, and to a deepened understanding of the mechanisms of the reactions utilized.

Cooperation with the colleagues in India in the field of chemistry of morphine and codeine is continuing.

In cooperation with Professor Merlini and his associates the earlier studies on the chemistry of powerfully photosensitizing perylenequinones from certain molds has been continued. The complex stereochemistry of these mold metabolites presents unusual problems.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-AM-29005-10-LCP

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Asymmetric synthesis; structure, stereochemistry and NMR

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI :	Herman Ziffer	Research Chemist	LCP, NIADDK
OTHERS :	Satoru Ito	Visiting Fellow	LCP, NIADDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Section on Molecular Biophysics

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In exploring the scope and limitations of using the mold, *Rhizopus nigricans*, for the hydrolysis of racemic acetates to prepare chiral alcohols, we examined the products from the hydrolysis of a series of medium-ring 2-cycloalkenyl acetates. These substrates were chosen because of their similarity to a variety of plant and marine natural products. In order to increase the versatility of these chiral alcohols as synthetic intermediates, the 2-bromo derivatives, and both (*E*) and (*Z*) isomers of the parent allylic alcohols were employed as substrates for *R. nigricans*. In every case, except for 2-cyclodecenol, a chiral alcohol was formed and its absolute stereochemistry was established as *R* by chemical transformation into the corresponding dimethyl α-acetoxydicarboxylate of known absolute stereochemistry. The chiral alcohols were then used to determine whether a recently proposed rule by Harada and Nakanishi, relating the signs of the c.d. bands of *p*-substituted benzoates of allylic alcohols with their absolute stereochemistry, could be employed for medium-ring allylic alcohols. These compounds exist in many more conformations than are available for the five-, six- and seven-membered analogues. Although the signs of the c.d. bands of p-bromobenzoates of the medium-ring esters differed from those of the five-, six- and seven-membered analogues it was possible to rationalize the change in sign with the Harada-Nakanishi rule and with information on the conformation calculated as the most stable for each ring system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AM-29006-14-LCP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The structure and dynamics properties of macromolecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

P.I.: Elliot Charney, Research Chemist, LCP-NIADDK

Others: Donald C. Rau, Senior Staff Fellow, LCP-NIADDK
Shousun Szu, Senior Staff Fellow, LCP-NIADDK

COOPERATING UNITS *(if any)* E. Appella, NCI:LCB; H-H. Chen, George Mason University, Fairfax, VA; Martin Gellert, A:LMB; D. Hansburg, Fox Chase Cancer Center, Philadelphia, PA; E. Henry, A:LCP; A. Maxwell, A:LMB; V. A. Parsegian, DCRT; J. Sussman, Weizmann Institute of Science, Rehovot, Israel

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Spectroscopy and Structure

INSTITUTE AND LOCATION

NIH, NIADDK, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A. Macromolecular structure, dynamics and polyelectrolyte propeties of large biological polymers, in particular, polynucleotides and nucleic acids are being studied by electric-field induced dichroism and birefringence methods. Theoretical and computational methods supplement the experimental work.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AM-29007-13-LCP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure and interaction of biomolecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Hideo Kon, Research Chemist, LCP-NIADDK

Others: Kazunori Kon, Visiting Fellow, LCP-NIADDK

COOPERATING UNITS *(if any)*

Tsuneo Takahashi (American Red Cross)
Eugene O'Bryan (CSL-DCRT)

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Section on Spectroscopy and Structure

INSTITUTE AND LOCATION

NIH, NIADDK, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 2	PROFESSIONAL: 2	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have applied the spin label ESR method for studying deformation and orientation of human erythrocytes in shear flow, and demonstrated i) the effect of the cell-cell interaction, which we attributed to the effective size of the cell in flow, and ii) the effect of the presence of hardened cell in a mixed suspension on the average deformation and orientation of the intact cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AM-29008-13-LCP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Electronic and molecular structural investigations.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Ruth McDiarmid, Research Chemist, LCP-NIADDK

Others: Aleksandar Sabljic, Visiting Fellow, LCP-NIADDK

COOPERATING UNITS *(if any)*

John Doering - Johns Hopkins University
Leo Klasinc - Rudjer Boskovic Institute, Yugoslavia

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Spectroscopy and Structure

INSTITUTE AND LOCATION

NIH, NIADDK, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☐ (a)[1] Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1. Discrepancies previously observed between the spectra of butadiene and 2,3-dimethylbutadiene around the second valence transition have been demonstrated to arise from a thermally induced enhancement of this forbidden valence transition.

2. The barrier to methyl torsion in 2,3-dimethylbutadiene has been shown to be due predominantly to the out-of-plane pi electron orbitals of the molecule.

3. The valence spectra of cis and trans hextrienes have been studied. In each molecule, the allowed valence transition is lowest in energy. The first singlet and triplet transitions occur at the same energies in the two isomers. Both the second singlet and triplet transitions are higher in energy in the trans molecule than in the cis molecule.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AM-29009-11-LCP

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies on Sickle cell disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: William A. Eaton, Medical Officer, LCP-NIADDK

Others: James Hofrichter, Research Chemist, LCP-NIADDK

COOPERATING UNITS (if any)

Frank A. Ferrone, Assistant Professor, Department of Physics, Drexel University

LAB/BRANCH
Laboratory of Chemical Physics

SECTION
Macromolecular Biophysics

INSTITUTE AND LOCATION
NIH, NIADDK, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 0.75	PROFESSIONAL: 0.75	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The thermodynamics and kinetics of hemoglobin S polymerization are being investigated in order to elucidate the molecular mechanism for this process. The results of these studies provide the rationale for developing agents that can be used in the treatment of patients with sickle cell disease. A method to measure the distribution of delay times for intracellular polymerization is being developed as an assay technique to test the efficacy of potential therapeutic agents in inhibiting intracellular polymerization of hemoglobin S.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AM-29010-12-LCP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Conformation and electronic structure of biological molecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: William A. Eaton, Medical Officer, LCP-NIADDK

Others:
James Hofrichter, Research Chemist, LCP-NIADDK
Eric R. Henry, Senior Staff Fellow, LCP-NIADDK
Robert Deutsch, Staff Fellow, LCP-NIADDK

COOPERATING UNITS *(if any)*

Takashi Yonetani, Professor, Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, Philadelphia, PA

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Macromolecular Biophysics

INSTITUTE AND LOCATION

NIH, NIADDK, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.2	1.7	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A transient laser spectrometer has been developed for measurements of optical absorption spectra and kinetics of hemoglobin conformational changes and ligand rebinding following photolysis of the carbon monoxide complex. Molecular dynamics calculations are being carried out to simulate the heme and globin structural changes and the ligand motion following photolysis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AM-29011-13-LCP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

The physics and chemistry of photoreception

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator*) (*Name, title, laboratory, and institute affiliation*)

PI:	William A. Hagins	Medical Officer	A:LCP
Others:	S. Yoshikami	Research Biologist	A:LCP
	F. M. Hagins	Guest Worker	A:LCP
	M. C. Foster	Special Expert	A:LCP
	P. Rashidian	Electronics Engineer	A:LCP

COOPERATING UNITS (*if any*)

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Section on Membrane Biophysics

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 2	PROFESSIONAL: 2	OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

An investigation of the mechanics of phototransduction in vertebrate photoreceptor cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AM-29012-14-LCP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The influence of molecular structure on chemical and biological properties

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	N. E. Sharpless	Research Chemist	A:LCP
Others:	R. G. Adams	Research Physicist	A:LCP
	W. H. Jennings	Research Physicist	A:LCP
	K. Sollner	Scientist Emeritis	A:LCP

COOPERATING UNITS *(if any)*

Peter F. Kador, EI, LVR, NIH

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Section on Membrane Biophysics

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Energy minimization calculations and quantum mechanical computations on compounds of biological and pharmacological interest continue to give insight into and explanations of their modes of behavior, resulting in clues to their pharmacophores.

The inhibition of the enzyme, aldose reductase, by a wide variety of compounds continues under investigation by QSAR techniques as well as by energy minimization computations, quantum mechanics and stereochemical considerations. A model for the interaction of a wide range of types of compounds with aldose reductase has been proposed and their common features attained. Preliminary circular dichroism studies on the enzyme itself seem to indicate the enzyme to be an α protein.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AM-29015-13-LCP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Digital computer facilities for LCP and LMB

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)*

PI: W. H. Jennings, Jr. Research Physicist A:LCP

COOPERATING UNITS *(if any)*

Computer Systems Laboratory, DCRT: A. R. Schultz, Jr., J. I. Powell, E. O'Bryan, D. C. Carpenter

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Section on Membrane Biophysics

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The laboratory computer facility serving LCP and LMB was in routine operation during the reporting period. This local network services twelve laboratory instruments with dedicated microcomputer systems which are connected to a large host computer by a store-and-forward communications processor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AM-29016-09-LCP

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Macromolecular dynamics and assembly reactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: James Hofrichter, Research Chemist, LCP-NIADDK

Others: William A. Eaton, Medical Officer, LCP-NIADDK
Eric R. Henry, Senior Staff Fellow, LCP-NIADDK
Robert Deutsch, Staff Fellow, LCP-NIADDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Spectroscopy and Structure

INSTITUTE AND LOCATION

NIH, NIADDK, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL:	OTHER
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Transient spectroscopy has been used to study the photo-induced dissociation of carbon monoxide from hemoglobin. The data provide a detailed description of the pathway for ligand dissociation, revealing three distinct structural relaxations in the unliganded protein. Experiments on hybrid iron-cobalt hemoglobin show spectral changes in the cobalt hemes which can be used to determine how rapidly structural changes initiated by photolysis of CO from the Fe hemes are propagated to other subunits. Effort has been focussed on interpreting the spectral changes observed in these experiments. To compare spectral changes from molecules in which the ligand-rebinding kinetics to the Fe hemes are different, the rebinding curves must be known accurately. An improved analysis of ligand binding has been developed which permits us to compare spectral changes for the hybrids and normal hemoglobin and resolve the spectral changes from the Co subunits.

When the delay time for polymerization of sickle cell hemoglobin exceeds 1 sec the kinetics of polymerization become stochastic when the reaction is monitored in very small volumes ($<10^{-10}$cc). The distribution of delay times can be measured by performing several hundred identical experiments. During the past year a simple theory has been developed which permits these distributions to be analyzed to give the homogeneous nucleation rate and other parameters of the dual-nucleation model proposed by Ferrone et al., (1980). Interpretation of these distribution permits us to evaluate the temperature dependence of the homogeneous nucleation rate and to obtain direct estimates of the size of the nucleus. The results supplement the analysis of bulk experiments using the same model which has been completed in collaboration with Dr. Ferrone. Together, these two studies show that this model provides an excellent description of the nucleation controlled polymerization of hemoglobin S.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AM-29017-05-LCP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Spectroscopic investigation of membrane lipids and models

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI :	Ralph G. Adams	Research Physicist	LCP, NIADDK
OTHERS:	Ira W. Levin	Research Chemist	LCP, NIADDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Section on Molecular Biophysics

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Infrared and Raman spectroscopic studies have been used to characterize conformational changes in the gel and liquid crystalline forms of simple model membrane systems. Schemes were devised for determining reliable, integrated intensities of the C-H stretching mode region of lipid bilayers using a Raman spectrograph coupled to a multichannel diode array detector. This intensity probe is particularly sensitive to bilayer expansion effects, as, for example, the structural changes involved in the membrane pretransition.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AM-29019-04-LCP

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Theoretical studies on the dynamic aspects of macromolecular function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. Szabo	Research Chemist	A:LCP
Others:	G. Lamm	Staff Fellow	A:LCP

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemical Physics

SECTION

Macromolecular Biophysics

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2	2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Theoretical approaches are being developed to extract information concerning the nature of internal motions in a variety of biopolymers from nuclear magnetic relaxation and time-resolved fluorescence depolarization experiments. In order to interpret fluorescence studies where the time dependence of the anisotropy depends on excitation frequency and/or the total intensity is multiexponential, a comprehensive formalism has been developed that treats both excited state and orientation dynamics in a unified way. This approach readily handles not only overall and internal reorientation of probes but also such complications as energy transfer, heterogeneity and the interconversion of excited states with different emission characteristics. The role of diffusion in reaction dynamics and electrochemical processes has been investigated. A new approach has been developed to treat localized traps in diffusion processes and has been used to establish the relationship between the time-dependent germinate recombination yield and the bimolecular rate in diffusion influenced reactions. Novel Monte Carlo Brownian dynamics and finite difference algorithms have been developed and applied to the solution of realistic diffusion problems including the simulation of the current to microelectrodes that are used *in vivo* studies of the concentration of electroactive molecules.

ANNUAL REPORT OF THE LABORATORY OF BIOORGANIC CHEMISTRY

NATIONAL INSTITUTE OF ARTHRITIS, DIABETES, DIGESTIVE AND KIDNEY DISEASES

SECTION ON NEUROBIOLOGY

Receptors for Neurotransmitters and Drugs in the Central Nervous System

Previous studies from this laboratory have shown that a novel series of "annelated" 1,4-diazepines ("hetrazepines") have high affinities for "brain-type" benzodiazepine receptors *in vitro* and possess anticonflict and anticonvulsant but not hypnotic activity *in vivo*. In contrast to classical 1,4-benzodiazepines (e.g. diazepam), the apparent affinities of these compounds were not increased by γ-aminobutyric acid (GABA) under a variety of experimental conditions. These observations invalidate the proposed *in vitro* procedure reported by this laboratory and others (1982) of a facile *in vitro* assay to discriminate benzodiazepine "agonists" from "antagonists". The electrophysiological actions of several hetrazepines have now been examined in rat substantia nigra zona reticulata, an area of the brain previously reported to be responsive to both benzodiazepines and γ-aminobutyric acid. WE 973 and WE 1008 (two hetrazepines that do not appear to possess hypnotic qualities and do not have their apparent affinities altered by GABA) inhibit the spontaneous firing of these neurons. However, in contrast to "classical" 1,4-benzodiazepines and a hypnotic hetrazepine (brotizolam; WE 941), neither WE 973 nor WE 1008 augmented the inhibition of cell firing elicited by iontophoretic application of GABA. These results strongly suggest that direct occupation of benzodiazepine receptors by high affinity ligands may be a sufficient condition to elicit an anticonflict and anticonvulsant action, and that the well described augmentation of GABAergic transmission by classical 1,4-benzodiazepines may be responsible for a hypnotic action.

Previous studies from this laboratory have established that injection of certain C-3 substituted β-carbolines represents a robust, chemically induced model of anxiety in primates. Several compounds which possess anticonflict or anxiolytic actions but do not interact with benzodiazepine receptors *in vitro* have now been examined in this model. The serotonin antagonist cyproheptadine was shown to block some, but not all, of the repertoire of behaviors produced by injection of 3-carboethoxy-β-carboline (β-CCE). However, cyproheptadine was unable to reverse the somatic changes (e.g. increases in heart rate and blood pressure) produced by β-CCE. Although cyproheptadine antagonized β-CCE induced increases in plasma cortisol and ACTH, it was unable to antagonize increases in plasma norepinephrine and epinephrine induced by β-CCE. A non-sedating dose (1 mg/kg, i.v.) of the GABAmimetic THIP displayed a markedly different profile against β-CCE. Some, but not all of the behaviors produced by β-CCE were antagonized by THIP. THIP was able to antagonize the increase in heartrate produced by β-CCE but not the increase in blood pressure. Further, THIP did not block increases in plasma ACTH, partially antagonized the rise in plasma norepinephrine, but was unable to antagonize the rise in plasma epinephrine produced by β-CCE. Thus, studies with a number of "atypical" anxiolytic or anticonflict agents including propranolol, clonidine, cyproheptidine, and THIP suggest that an anxiolytic action may be produced through activation of several neurochemical pathways or through a peripheral mechanism (e.g. with propranolol) bypassing the benzodiazepine-GABA receptor-chloride ionophore complex.

Numerous clinical studies have demonstrated an association between the development of depressive states and anxiety. Since administration of β-carbolines appears to be a chemically induced model of anxiety, we examined the ability of an anxiogenic β-carboline (FG 7142) to induce "learned helplessness" behavior (thought to be an animal model of depression) in rodents. A single injection of FG 7142 (10 mg/kg) was shown to be behaviorally "equivalent" to 60-90 minutes of uncontrolled tail shock in producing learned helplessness behavior in rats within 24 hours after exposure to the drug. Furthermore, the effects of FG 7142 could be prevented by the administration of the benzodiazepine antagonist Ro 15-1788 which suggests the effects of FG 7142 are produced via benzodiazepine receptors. These studies provide an intriguing animal model linking anxiety with depression.

The observation that barbiturates (e.g. pentobarbital) are capable of enhancing the apparent affinity of benzodiazepine receptor ligands resulted in a study concluding that the anticonflict actions of pentobarbital may be indirectly mediated through benzodiazpine receptors. Both *in vivo* and *in vitro* data suggest other pharmacologic and toxicologic actions of barbiturates might also be mediated through the benzodiazepine-GABA receptor chloride ionophore complex. Barbiturates such as pentobarbital have been shown to bind to a site or near the chloride ionophore (demonstrated using both [^{3}H]dihydropicrotoxin and [^{35}S]t-butylbicyclophosphorothionate (TBPS)). Thus, a search was initiated for compounds which could reverse the toxic actions of pentobarbital using the abilities of these substances to act at either the benzodiazepine receptor or chloride ionophore as "leads". It was found that isopropylbicyclophosphate (IPPO), a "cage convulsant" which binds at or near the chloride ionophore, greatly reduced the overall mortality of animals pretreated with a lethal dose of pentobarbital. Picrotoxin, which binds to the same site (and was formerly used as an antidote for barbiturate poisoning) also reduced pentobarbital lethality, but only at doses which were lethal when given alone. Thus, IPPO represents a prototype for development of a "molecular" antagonist of babiturate action. This observation suggests that compounds which have high affinities for the chloride ionophore *in vitro* could lead to the development of a specific antidote for clinical treatment of barbiturate toxicity.

Previous studies from this laboratory have shown that the target size of the benzodiazepine receptor (using [^{3}H]diazepam as a radioligand) is approximatley 54,000 daltons, while the target size of "peripheral-type" binding sites for benzodiazepines in kidney is approximately 30,000 daltons. Further studies using a benzodiazepine antagonist (Ro 15-1788) and an "active" antagonist (β-CCE) as radioligands demonstrated a target size of 51,000 ± 2,000. This observation has shown the target size of the benzodiazepine receptor is independent of the pharmacologic actions of the ligand used, and thus supports the "domain" hypothesis of agonist/antagonist interaction rather than the classical "state-transition" theory. Further, the target size for the GABA receptor linked to benzodiazepine receptors is identical to the benzodiazepine receptor. The target size of the chloride ionophore (estimated with [^{35}S]TBPS as a radioligand) was about three fold higher (138,000 daltons). These observations suggest that the GABA and benzodiazepine receptors are physically as well as functionally coupled, and may be physically distinct from the chloride ionophore.

Binding sites for benzodiazepines such as diazepam have also been described in peripheral tissues (e.g. kidney) and transformed cells of neural origin. These "peripheral-type" binding sites (PBS) for benzodiazepines are pharmacologically as well as physically distinct from the "brain-type" benzodiazepine receptors. We previously demonstrated that guinea pig brain is rich in PBS and that Ro 5-4864 (4'-chlorodiazepam), a ligand for this site (K_d ~1 nM), is a potent convulsant in the guinea pig. Ro 5-4864 has now been shown to be a potent convulsant in mice and rats as well as guinea pigs. Further, Ro 5-4864 increased cell firing in neurons of the substantia nigra zona reticulata at doses well below those needed to elicit convulsions. The pharmacologic profile of agents which are capable of antagonizing both the electrophysiologic and convulsant effects of Ro 5-4864 suggest that "brain-type" benzodiazepine receptors are not directly involved in these actions. However, these studies did not rule out the possibility that Ro 5-4864 (4'-chlorodiazepam) could be acting at another component of the "supramolecular complex".

Autoradiographic studies of the distribution of PBS in rat and guinea pig revealed a high site density in the pineal gland. Neurochemical and pharmacologic evaluation of PBS demonstrated a site density in pineal (> 24 pmol/mg protein) approximately 100 fold higher than most areas of brain and 10 fold higher than most peripheral tissues. Nonetheless, these sites appear to be pharmacologically similar to PBS in other tissues.

The highest density of PBS in rat brain was found in olfactory bulb. The prominent facial component of Ro 5-4864 induced convulsions, coupled with high site density in the olfactory bulb prompted an investigation of the effects of surgical isolation of the olfactoy bulbs on PBS density. A significant reduction in the density of PBS was observed 27-31 days after surgical isolation of the olfactory bulbs. No reduction in the density of cerebellar PBS was observed. A reduction in "brain-type" benzodiazepine receptors was also observed after surgical isolation of the olfactory bulbs, but this effect occurred 1-2 weeks prior to the reduction in PBS. These observations suggest that PBS in brain are under neural control and are not exclusively associated with non-neuronal elements as had been previously proposed. Furthermore, extirpation of the olfactory bulbs resulted in a significant reduction in the potency of Ro 5-4864 as a convulsant but did not reduce the potency of PTZ. These data suggest that PBS in the olfactory bulb may contribute to the convulsant actions of Ro 5-4864.

Despite the ubiquitous distribution of PBS, the physiologic role in peripheral tissues is still unknown. In the kidney, PBS appear to be localized in the thick ascending limb of Henle (TAL) and the distal convoluted tubule, where the osmolarity may be as high as 1200 mOsm. Thus, studies were intiated to determine whether PBS are involved in ion transport phenomena. Using several sodium and potassium salts, it was found that the apparent affinity of [^{3}H]Ro 5-4864 was reduced by some anions in crude homogenates of kidney. Iodide was the most powerful anion at reducing the binding of [^{3}H]Ro 5-4864 while sulfate ions were ineffective. Furthermore, 9-anthroic acid, DIDS and SITS (which affect chloride transport) also inhibited [^{3}H]Ro 5-4864 binding to kidney homogenates by 30-40%, while ouabain was ineffective at concentations of up to 1 mM. Binding of PK 11195, a quinoline derivative, which has a high affinity for PBS, was little affected by equivalent treatment with either 500 mM iodide or 1 mM DIDS. However, brief treatment of the crude membrane preparation with several detergents rendered PK 11195 susceptible to the influence of both iodide and DIDS. Thus, PBS may play a role in anion transport in the kidney.

Acidified methanol or trichloroacetic acid extraction of membranes from peripheral tissues or brain followed by ultrafiltration and/or gel filtration and high performance liquid chromatography revealed the presence of both high (Mr > 10,000 daltons) and low (Mr > 500 daltons) molecular weight substances which competitively inhibit the binding of [^{3}H]Ro 5-4864 to PBS. The binding of [^{3}H]diazepam to "brain-type" benzodiazepine receptors was only marginally reduced at concentrations of inhibitor which completely inhibited [^{3}H]Ro 5-4864 to PBS. Within the limited number of tissues surveyed, there appears to be an inverse relationship between the amounts of these endogenous inhibitors and the density of PBS in the same tissue. Separation of the high molecular weight fraction through reverse phase high performance liquid chromatography revealed three discrete regions of inhibitory activity, suggesting the high molecular weight fraction (obtained from a Sephadex G-50 column) consists of at least three components. The chemical nature and physiologic function of these substances remains to be elucidated.

Previous studies from this laboratory have demonstrated an augmentation of some of the behavioral actions of phencyclidine (PCP) by dihydropyridine (DHP) calcium channel antagonists such as nifedipine and nitrendipine. These observations prompted a series of *in vitro* studies to determine if a neurochemical correlate of this effect could be demonstrated. PCP was found to produce a dose dependent, biphasic alteration in [^{3}H]nitrendipine binding in brain. Low concentrations of PCP were found to enhance [^{3}H]nitrendipine binding while higher doses inhibited binding. Kinetic studies demonstrated that PCP altered the apparent affinity (Kd) of [^{3}H]nitrendipine but did not affect the B_{max}. This effect was dependent on the brain area used, and very low concentrations of Ca^{+2} were found to inhibit this enhancement of [^{3}H]nitrendipine binding by PCP. A number of PCP derivatives, as well as a number of compounds structurally unrelated to PCP (but which share pharmacologic properies with PCP) were examined for their effects on [^{3}H]nitrendipine binding. It was observed that PCP-like compounds could accommodate a relatively large degree of structural modification with little loss in efficacy. Further, compounds such as (±)SKF 10,047, levorphanol and dextrorphan also enhanced the apparent affinity of [^{3}H]nitrendipine. There was no correlation between the affinities of a series of compounds to enhance [^{3}H]nitrendipine binding and their affinities at "PCP receptors" previously described in brain. Thus, the augmentation of [^{3}H]nitrendipine binding may occur at a discrete locus.

Addition of an isothiocyanate function to the PCP molecule resulted in a compound which irreversibly inactivates [^{3}H]nitrendipine binding. At low concentrations (10^{-5} M), this compund produced an effect similar to PCP while higher concentrations (greater than 10^{-4} M) inhibited [^{3}H]nitrendipine binding.

Recently, dihydropyridine calcium channel "agonists", such as BAY K 8644, have been synthesized. Such compounds have been shown to enhance calcium flux in both smooth and cardiac muscle. We have investigated the behavioral actions of this compound in mice and have observed ataxia, decreased motor activity, Straub tail, arched back, limb clonus and tonus, and an increased sensitivity to auditory stimuli. These effects were observed at relatively low doses of (2-4 mg/kg, i.p.) of drug. The behavioral effects of BAY K 8644 wre selectively antagonized by the DHP calcium channel antagonist, nifedipine, which did not by itself produce behavioral changes. In contrast, the non-DHP calcium channel antagonist verapamil did not produce any alteration in BAY K 8644-induced behavior. The interaction of BAY K 8644 at the brain DHP binding site was confirmed by its potent competitive inhibition (Ki ~7 nM) of [^{3}H]nitrendipine binding. These data suggest that BAY K 8644 may produce its behavioral effects through an interaction with the DHP binding site in brain.

Autoradiographic studies have suggested that DHP binding sites are localized to discrete neuronal structures such as synaptic nerve endings and dendritic projections. We have studied the effects of various neurotoxins on the level of [^{3}H]nitrendipine binding in order to determine the functional significance of these sites in the CNS. Significant increases in the binding of [^{3}H]nitrendipine (25-30%) were observed after intraventricular administration of the catecholaminergic neurotoxin 6-hydroxydopamine in the hippocampus, but not cerebral cortex. In contrast, the serotonin neurotoxin 5,7-dihydroxytryptamine and the catecholamine depleting agent reserpine did not change the density of DHP binding sites. These findings suggest that a subpopulation of DHP binding sites are postsynaptically localized on catecholaminergic (noradrenergic) nerve terminals.

Previous studies from this laboratory have demonstrated the presence of high affinity, stereospecific binding sites for [^{3}H]methylphenidate in striatum and brainstem of the rat. Subsequent studies have demonstrated that the binding of [^{3}H]methylphenidate is localized in synaptosomes, and that the binding is dependent on the presence of Na^+. Intraventricular administration of 6-hydroxydopamine or medial forebrain bundle lesions resulted in a significant loss of [^{3}H]methylphenidate binding in striatum which was correlated with a loss in the capacity of this tissue to take up [^{3}H]dopamine. Structure-activity studies suggest that this site is associated with a dopamine transport system since a high correlation ($r = 0.88$, $p < .001$) was found between the potencies of a series of compounds to inhibit [^{3}H]dopamine uptake and inhibit [^{3}H]methylphenidate binding. These findings suggest the methylphenidate binding site may be part of a dopamine "transporter", analogous to findings demonstrating that [^{3}H]imipramine and [^{3}]desipramine label components of serotonin and dopamine "transporters", respectively. Further, pilot studies in human autopsy material suggest a distribution of site density similar to that found in rat, the highest site densities in striatum, and lowest in cerebellum.

Previous studies from this laboratory have shown that binding sites for [^{3}H]amphetamine in hypothalamus are reduced by 24 hours of food deprivation (30% reduction in B_{max}), and the site density restored to control levels if the animals are permitted to refeed for four hours. We have examined the factors responsible for this rapid modulation in site number, and have now demonstrated that intraperitoneal injection of D-glucose elicited significant increases in [^{3}H]amphetamine binding in the hypothalamus. This treatment did not alter [^{3}H]amphetamine binding in other brain areas. Injection of L-glucose failed to elicit this increase in [^{3}H]amphetamine binding. Further, injection of 2-deoxyglucose coupled with food deprivation elicited a similar increse in site density. However, if animals were permitted access to food during this four hour interval following injection of 2-deoxyglucose, the increase in [^{3}H]amphetamine binding was not observed. These observations suggest that [^{3}H]amphetamine binding sites in hypothalamus are coupled to glucose utilization and/or transport. The high correlation previously reported between the ability of a number of phenethylamines to inhibit [^{3}H]amphetamine binding and their potencies as anorectics may thus link the anorectic action of phenethylamines with their abilities to alter glucose utilization and/or transport.

SECTION ON OXIDATION MECHANISMS

Enzymatic Oxidation of Drugs to Toxic and Carcinogenic Metabolites

Previous annual reports from this Section have described a systematic approach to the study of the cytotoxicity, mutagenicity, and carcinogenicity of several polycyclic aromatic hydrocarbons. Briefly these studies have consisted of i) synthesis of as many known and potential oxidative metabolites as was possible, ii) study of the metabolism of the hydrocarbons with these authentic standards in hand, iii) testing these compounds for cytotoxic and mutagenic activity with bacterial and mammalian cells both in the presence and in the absence of added drug metabolizing systems such as cytochrome P-450 and epoxide hydrolase, and iv) evaluation of the carcinogenicity of these synthetic standards in several animal models. These studies provided evidence which indicated that bay-region diol epoxides are the most potent carcinogenic metabolites of these hydrocarbons. We formulated the "bay-region" theory which predicts that diol epoxides that have the epoxide group in the bay-region of the hydrocarbon will be the most chemically reactive and presumably biologically active diol epoxides from hydrocarbons that are tumorigenic. To date studies from our laboratory as well as several other laboratories around the world have either proved or implicated bay-region diol epoxides as ultimate carcinogens for benzo[a]pyrene, benz[a]anthracene, benz[c]acridine, 7-methylbenz[a]anthracene, benzo[b]fluoranthrene, 7,12-dimethylbenz[a]anthracene, 3-methylcholanthrene, dibenz[a,h]anthracene, two dibenzpyrenes, chrysene, 5-methylchrysene, benzo[c]phenanthrene, and certain methylated cyclopentaphenanthrenes. The theory has stimulated considerable research in the field, all of which has supported our initial concepts. Significant exceptions to the theory have yet to emerge.

One aspect of hydrocarbon-induced carcinogenesis which the bay-region theory made no attempt to take into account is that of relative and absolute stereochemistry of ultimate carcinogens. Many of our current efforts continue to address this question. Studies of the dihydrodiols and resultant bay-region diol epoxides formed from benz[a]anthracene as well as phenanthrene and chrysene by liver microsomes have shown that these molecules are all superimposable with the corresponding benzo[a]pyrene metabolites when their bay-regions are aligned. In the benzo[a]pyrene case, only one of four stereoisomeric bay-region 7,8-diol-9,10-epoxides exhibits strong tumorigenic activity, namely the predominant metabolically formed isomer. Tumor studies have shown that the related stereoisomer (R,S-diol-S,R-epoxide) is also the active form from chrysene and benz[a]anthracene. Tumor studies on the optically active benzo[c]phenanthrene 3,4-diol-1,2-epoxides are in progress. Results of the present studies are suggestive that there is a highly enantioselective site with which these carcinogens interact within the cell. Studies are in progress which will further define the steric constraints of the active site of cytochrome P-450c, the principal oxidative enzyme responsible for the conversion of polycyclic aromatic hydrocarbons to ultimate carcinogens.

Synthetic Studies. Optically active K-region arene oxides of known absolute configuration are of considerable interest in i) the study of the stereoselectivity of the cytochromes P-450 which form them as metabolites, ii) the study of their comparative mutagenic activity, and iii) the determination of the regiospecificity and kinetic properties of epoxide hydrolase and the glutathione S-transferases which act upon them. We have developed an efficient new synthesis of K-region arene oxides which is applicable to the preparation of optically active products and which is based on the finding that many polycyclic aromatic hydrocarbons react readily with N-bromoacetamide in acetic acid to form trans bromohydrin acetates at their K-regions. Treatment of the bromohydrin acetates with dry sodium methoxide leads directly to the arene oxides. Alternatively, the acetate can be replaced by a chiral ester for purposes of resolution and subsequent cyclization to the desired chiral arene oxide. Aza- as well as carbocyclic polycyclic aromatic hydrocarbons are amenable to this approach.

Solvolytic Reactivity of Diol Epoxides. Formulation of the bay-region theory has prompted substantial interest in the development of quantum mechanical models capable of predicting reactivity of bay-region diol epoxides. All of these calculational approaches have attempted to predict the ease of benzylic carbocation formation from bay-region diol epoxides of different polycyclic aromatic hydrocarbons since this reaction should be one of the factors which influence biological activity. It should be possible to corroborate quantitatively the validity of such calculational approaches by observations of the chemical reactivity of the diol epoxides in a well defined system. We have continued to study the solvolytic rates of diol epoxides to obtain a data base for comparison with various computational parameters such as the following reactivity indices: $\Delta E_{deloc}/\beta$, from perturbational molecular orbital calculations, $\Delta E_{\pi}/\beta$, from Huckel molecular orbital calculations, log (SC ratio) from resonance theory and ΔE values (relative to dibenzo[a,i]pyrene) from SCF INDO calculations. Our recent finding (see below) that reactivity of diol epoxides in pH-independent hydrolysis reactions is strongly influenced by the preferred conformation of the diol epoxides means that the reactivity of diol epoxides cannot be predicted by quantum-mechanical models that are based solely on the electronic properties of the aromatic ring systems.

Kinetics of the hydronium ion catalyzed (k_H) and pH-independent (k_o) hydrolyses of several benzo-ring diol epoxides, derived from polycyclic aromatic hydrocarbons, that possess bay-region trans diol groups have been investigated in 1:9 dioxane-water at 25°C. These epoxides are 1,2,3,4-tetrahydrobenz[a]anthracene-1,2-diol 3,4-epoxide, 1,2,3,4-tetrahydrotriphenylene-1,2-diol 3,4-epoxide, and 9,10,11,12-tetrahydrobenzo[e]pyrene-9,10-diol 11,12-epoxide. Both possible diastereomers of the diol epoxides in which the two hydroxyl groups are trans to each other were investigated: isomer 1, in which the benzylic hydroxyl group is cis to the epoxide oxygen, and isomer 2, in which this hydroxyl group and the epoxide oxygen are trans. The corresponding tetrahydro epoxides that lack a diol group were also investigated. For comparison, isomers 1 and 2 of 1,2,3,4-tetrahydrobenz[a]anthracene-3,4-diol 1,2-epoxide, a bay-region epoxide with a non-bay-region trans diol group, and the corresponding benz[a]anthracene tetrahydro epoxide were also studied. The most striking feature of the reactions of the diol epoxides that possess a bay-region diol group is a reversal of the relative reactivity of isomers 1 and 2 in the k_o reaction, when compared with diol epoxides whose diol group is not in a bay region. This reversal of reactivity, which causes k_o to be larger for isomer 2 than for isomer 1 when the diol is in a bay region, is explained by changes in conformational equilibria involving the cyclohexene ring due to steric crowding of the bay-region diol. Preference of this diol group for a pseudodiaxial conformation favors a conformation of isomer 2 in which the benzylic C-O bond of the epoxide is more or less aligned with the π orbitals of the aromatic system and strongly disfavors this aligned conformation of isomer 1. Reaction via the k_o process is faster for the aligned than for the nonaligned conformer; thus for epoxides with bay-region diol groups, k_o for isomer 2 is faster than k_o for isomer 1. The pH-independent reaction of isomer 2 of 1,2,3,4-tetrahydrobenz[a]anthracene-1,2-diol 3,4-epoxide via the aligned conformer gives, in addition to cis and trans tetraol products, substantial quantities of a keto diol, whereas no keto diol was detected from the corresponding isomer 1. This also represents a reversal of the pattern of product formation generally observed with diol epoxides that lack a bay region in the vicinity of the diol group. Rate constants for hydronium ion catalyzed hydrolysis (k_H) are much less sensitive to conformational factors than k_o. The distribution of cis and trans tetraol products from hydronium ion catalyzed hydrolysis of the 1,2,3,4-tetrahydrobenz[a]anthracene-1,2-diol 3,4-epoxides and the 1,2,3,4-tetrahydrotriphenylene-1,2-diol 3,4-epoxides can be explained by preferential pseudoaxial attack of water upon the benzylic cations formed from these epoxides. On the basis of these observations and previous findings with non-bay-region diol, bay-region epoxides, rules for predicting the effects of conformation on rates and products of diol epoxide hydrolyses have been proposed.

Potential Antitumorigenic Agents. Substantial effort has been committed to the identification of compounds capable of blocking the biological activity of bay-region diol epoxides through their chemical inactivation. Ideally, such compounds should have a high affinity for the diol epoxide such that an initial non-covalent complex forms. Subsequent covalent interaction of the compound with the diol epoxide or general acid catalyzed hydrolysis of the diol epoxide to tetraols would lead to the desired biological result. Inhibition of diol epoxide induced mutagenicity has been used as a screening method to search for active compounds. Initially we had described the highly inhibitory activity of riboflavin 5'-phosphate (FMN) and ellagic acid. These studies have been continued.

Myricetin, robinetin and luteolin inhibited the mutagenic activity resulting from the metabolic activation of benzo[a]pyrene and (±)-trans-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene by rat liver microsomes. These naturally occurring plant flavonoids and seventeen additional flavonoids and related derivatives with phenolic hydroxyl groups inhibited the mutagenic activity of (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (B[a]P 7,8-diol-9,10-epoxide-2), which is an ultimate mutagenic and carcinogenic metabolite of benzo[a]pyrene. Several flavonoids without phenolic hydroxyl groups or with methylated phenolic hydroxyl groups were inactive. The mutagenic activity of 0.05 nmol of BP 7,8-diol-9,10-epoxide-2 towards strain TA 100 of *S. typhimurium* was inhibited 50% by incubation of the bacteria and the diol epoxide with myricetin (2 nmol), robinetin (2.5 nmol), luteolin (5 nmol), quercetin (5 nmol), 7-methoxyquercetin (5 nmol), rutin (5 nmol), quercitrin (5 nmol), delphinidin chloride (5 nmol), morin (10 nmol), myricitrin (10 nmol), kaempferol (10 nmol), diosmetin (10 nmol), fisetin (10 nmol), or apigenin (10 nmol). Considerably less antimutagenic activity was observed for dihydroquercetin, naringenin, robinin, D-catechin, genistein, kaempferide and chrysin. Pentamethoxyquercetin, tangeretin, nobiletin, 7,8-benzoflavone, 5,6-benzoflavone, and flavone, which lack free phenolic groups, were inactive. The antimutagenic activity of hydroxylated flavonoids results from their direct interaction with B[a]P 7,8-diol-9,10-epoxide-2 since the rate of disappearance of the diol epoxide from cell-free solutions in 1:9 dioxane:water was markedly stimulated by myricetin, robinetin and quercetin. Myricetin was a highly potent inhibitor of the mutagenic activity of bay-region diol epoxides of benzo[a]pyrene, dibenzo[a,h]pyrene and dibenzo[a,i]pyrene, but higher concentrations of myricetin were needed to inhibit the mutagenicity of the chemically less reactive benzo[a]pyrene 4,5-oxide and bay-region diol epoxides of benz[a]anthracene, chrysene and benzo[c]phenanthrene.

Biological Activity of Optically Active Benz[a]anthracene Metabolites. Previous reports have documented that the 3,4-dihydrodiol of benz[a]anthracene is a metabolite and proximate carcinogen of the hydrocarbon in accord with predictions of the bay-region theory. Current studies have examined the role of absolute configuration in the expression of mutagenic and tumorigenic activity of the bay-region benz[a]anthracene 3,4-diol-1,2-epoxides with the outcome that, like B[a]P and chrysene, the (4R,3S)-diol-(2S,1R)-epoxide isomer is the diol epoxide metabolite that has nearly all of the tumorigenic activity.

Enantiomers of the diastereomeric pair of bay-region benz[a]anthracene 3,4-diol-1,2-epoxides in which the benzylic 4-hydroxyl group and epoxide oxygen are either cis (isomer 1) or trans (isomer 2) were evaluated for mutagenic activity in two histidine-dependent strains of *Salmonella typhimurium*, as well as in an 8-azaguanine-sensitive Chinese hamster cell line. In strain TA 98 of *S. typhimurium*, the diol epoxide with (1S,2R,3R,4S) absolute configuration [(-)-diol epoxide 2] was the most active isomer, although there was less than a 3-fold difference in the mutagenicity of the four diol epoxides. However, in strain TA 100 of *S. typhimurium*, the enantiomeric diol epoxide with (1R,2S,3S,4R) absolute configuration [(+)-diol epoxide 2] was the most active diol epoxide, and the two isomers with (3S,4R) absolute configuration [(-)-diol epoxide 1 and (+)-diol epoxide 2] were three to eight times more active than were the two isomers with (3R,4S) configuration. The highest degree of sensitivity to absolute configuration was observed in Chinese hamster V79 cells, in which the (1R,2S,3S,4R) isomer [(+)-diol-epoxide 2] was from three to 20 times more mutagenic than were the other three isomers. This metabolically predominant (+)-diol epoxide 2 isomer, which has high activity in strain TA 100 of *S. typhimurium* and the Chinese hamster V79 cells, has the same absolute configuration as do the bay-region diol epoxide isomers of benzo[a]pyrene and chrysene that have been shown previously to be exceptionally mutagenic to mammalian cells and highly tumorigenic in mice. Analysis of the mutagenic activity of the (+)- and (-)-isomers of the 1,2- and 3,4-tetrahydroepoxides of benz[a]anthracene revealed only small enantiomeric differences in strain TA 98 of *S. typhimurium* (2.5-fold) and little, if any, differences (<1.5-fold) in the other two mutagenicity systems. However, the extent to which the four tetrahydroepoxides were converted to nonmutagenic products by homogeneous microsomal epoxide hydrolase (EC 3.3.2.3) indicated marked differences in the stereoselectivity of the enzyme. (-)-(3R,4S)-Epoxy-1,2,3,4-tetrahydrobenz[a]anthracene appears to be an exceptionally good substrate for epoxide hydrolase.

The tumorigenicity of the (+)- and (-)-enantiomers of the diastereomeric bay-region benz[a]anthracene 3,4-diol-1,2-epoxides was evaluated in two mouse tumor models. In an initiation-promotion experiment on mouse skin, a single topical application of 0.1 or 0.4 µmol of the benz[a]anthracene diol epoxides was followed by 25 weeks of promotion with 12-O-tetradecanoylphorbol-13-acetate. Of the four isomers of the bay-region diol epoxides, only (+)-(1R,2S,3S,4R)-3,4-dihydroxy-1,2-epoxy-1,2,3,4-tetrahydrobenz[a]anthracene [(+)-diol epoxide-2] and (+)-(1R,2S,3R,4S)-3,4-dihydroxy-1,2-epoxy-1,2,3,4-tetrahydrobenz[a]anthracene [(+)-diol epoxide 1] had significant tumor-initiating activity. (+)-Diol epoxide-2 was approximately 4-fold more active as a tumor initiator on mouse skin than was (+)-diol epoxide-1 at both doses tested. In newborn mice, a total of 0.14 µmol of compound, divided into three doses, was administered i.p. on the first, eighth, and fifteenth day of life, and tumorigenic activity was determined when the mice were 26 to 32 weeks of age. As was observed in the initiation-promotion experiment on mouse skin, only two of the four optical isomers of the bay-region diol epoxides produced a significant tumor incidence. (+)-Diol epoxide-2 induced a 100% incidence of lung tumors, with an average of 23.11 tumors/mouse, and was at least 60-fold more active (average number of tumors per mouse) than was (+)-diol epoxide-1, which produced a 31% lung tumor incidence and 0.38 lung tumors/mouse. (+)-Diol epoxide-2 was the only optical isomer that induced a significant incidence of hepatic tumors in male mice (31% incidence, 1.17 tumors/mouse). The highly tumorigenic (+)-diol epoxide-2 isomer with

(R,S,S,R) absolute configuration has the same absolute configuration as does the highly tumorigenic isomer of the bay-region diol epoxides of benzo[a]pyrene and chrysene.

Biological Activity of Benzacridine Derivatives. The mutagenic activities of benz[a]acridine, benz[c]acridine, and a number of their derivatives, including 12 epoxides and diol epoxides, were examined in bacterial and mammalian cells to determine the importance of bay-region activation of aza-polycyclic aromatic hydrocarbons. In *Salmonella typhimurium* strain TA 98, the diastereomeric bay-region 3,4-diol-1,2-epoxides of benz[c]acridine, in which the benzylic hydroxyl group is either cis (diol epoxide 1) or trans (diol epoxide 2) to the epoxide oxygen, had equivalent mutagenic potency (250 to 300 His^+ revertants/nmol), while in *S. typhimurium* strain TA 100, diol epoxide 1 induced 5100 His^+ revertants/nmol and was approximately twice as active as was diol epoxide 2. In Chinese hamster V79 cells, the diol epoxide 2 isomer of benz[c]acridine 3,4-diol-1,2-epoxide was approximately twice as mutagenic (4.5 8-azaguanine-resistant colonies/10^5 surviving cells/nmol) as diol epoxide 1. In all three test systems, the bay-region diol epoxides of benz[c]acridine were from 1 to 4 orders of magnitude more mutagenic than were the non-bay-region diol epoxides in which the epoxide was in the 3,4-, 8,9-, or 10,11-positions of the molecule. With the benz[a]acridine diol epoxides, the 3,4-diol-1,2-epoxide 2 diastereomer was 2 to 4 times more active than was the 3,4-diol-1,2-epoxide 1 diastereomer in the bacterial systems, but both diastereomers had less than 10% of the activity of their benz[c]acridine counterparts. The bay-region tetrahydro-1,2-epoxides of both benzacridines were exceptionally mutagenic, and the benz[c]acridine epoxide was 4- to 11-fold more active than was the benz[a]acridine epoxide. The bay-region diol epoxide diastereomers of benz[c]acridine and its bay-region tetrahydroepoxide were not metabolized to nonmutagenic products by highly purified epoxide hydrolase (EC 3.3.2.3). Metabolic activation experiments using the cytochrome P-450-dependent monooxygenase system and the dihydrodiol precursors of the bay-region diol epoxides were consistent with the intrinsic mutagenicity data, in that benz[c]acridine 3,4-dihydrodiol was metabolized to mutagenic products to a greater extent than was benz[a]acridine 3,4-dihydrodiol. The benz[c]acridine 1,2-, 5,6-, 8,9-, and 10,11-dihydrodiols were not metabolically activated to mutagenic metabolites. These results provide initial evidence that the bay-region theory can be extended to certain aza-polycyclic aromatic hydrocarbons and indicate that the position of the nitrogen heteroatom can markedly affect mutagenic activity.

Benz[c]acridine (B[c]ACR) and 12 of its derivatives, including the 5 metabolically possible trans-dihydrodiols, the diastereomeric bay-region diol epoxides, 2 non-bay-region diol epoxides, and the K-region arene oxide, were tested for tumor-initiating activity on mouse skin. A single topical application of 0.4 to 2.5 µmol of compound was followed 12 days later by twice-weekly applications of the tumor promoter 12-O-tetradecanoylphorbol-13-acetate for 25 weeks. B[c]ACR was a weak tumor initiator on mouse skin, producing a 37% tumor incidence and 1.33 tumors/mouse at the 2.5-µmol dose. Of the five metabolically possible trans-dihydrodiols of B[c]ACR, only trans-3,4-dihydroxy-3,4-dihydro-B[c]ACR had significant tumor-initiating activity. This compound was at least 6-fold more active than was the parent compound at the three doses tested. The diastereomeric bay-region diol epoxides, in which the epoxide oxygen is either cis (isomer 1) or trans (isomer 2) to the benzylic hydroxyl group, each had significant tumor-initiating activity, although isomer 2 was at least 5-fold more active than was isomer 1 and had activity equal to that of its potential metabolic precursor, trans-3,4-dihydroxy-3,4-dihydro-B[c]ACR. Two non-bay-region diol epoxides (isomer 2 of the 8,9-diol-10,11-epoxide and the 10,11-diol-8,9-epoxide) and the 5,6-arene oxide (K-region) were inactive at the doses tested. 3,4-Dihydro-B[c]ACR, the potential metabolic precursor of a bay-region tetrahydroepoxide, was the most potent tumor initiator analyzed in the present study. At an initiating dose of 0.4 µmol, this compound produced a 97% tumor incidence and 7.90 tumors/mouse after 15 weeks of promotion with 12-O-tetradecanoylphorbol-13-acetate. These results suggest that B[c]ACR, the N-12 analogue of benz[a]anthracene, undergoes metabolic activation to an ultimate carcinogenic metabolite via formation of a bay-region diol epoxide, as has already been demonstrated for benz[a]anthracene.

Effects of Substituents on Tumorigenicity. The tumorigenic activities of benzo[a]pyrene (BP) and 6-fluorobenzo[a]pyrene (6-F-BP) were compared to determine whether an unsubstituted 6-position is important for the carcinogenic effect of BP. Highly purified samples of 6-F-BP and BP had similar activities for the induction of lung adenomas in Swiss Webster mice treated before weaning. The 6-fluoro derivative, however, had about one-half as much activity as BP for the initiation of skin papillomas in CD-1 mice. Similarly, 6-F-BP (≈90% purity) had about one-half the activity of BP for the induction of skin tumors in C57BL/6J mice given repetitive treatments of the hydrocarbons and for the induction of sarcomas in C3H/fCum mice given a single sc injection. 6-F-BP (≈90% purity) had activity similar to that of BP for induction of sarcomas at the sc injection site in Fischer 344 rats. These results and related data indicate the need for detailed metabolic studies whenever fluorine substitution is used as a probe to assess the role of the unsubstituted position in the carcinogenicity of the parent compound.

3,6-Dimethylcholanthrene (3,6-DMC) and 7,11,12-trimethylbenz[a]anthrene (7,11,12-TMBA) were tested for tumor-initiating activity on mouse skin as an approach to evaluate the potential role of steric strain in the bay-region on tumorigenic potency. Methyl-substitution at the 6-position of 3-methylcholanthrene (3-MC) increases steric strain in the bay-region of the hydrocarbon as it does at the 12-position of benz[a]anthracene (BA) causing both hydrocarbons to become non-planar. 3,6-DMC had at least 2- to 3-fold higher tumor-initiating activity than did 3-MC. Introduction of an 11-methyl group in 7,12-dimethylbenz[a]anthracene (7,12-DMBA) results in the formation of a more highly hindered (buttressing effect) hydrocarbon. 7,11,12-TMBA had 5% or less of the tumor-initiating activity of 7,12-DMBA, although the hydrocarbon still had relatively high tumorigenic activity on mouse skin. The results obtained with 3,6-DMC and studies reported previously with other methyl-substituted hydrocarbons, show that hydrocarbons possessing steric strain in the bay-region of the molecule can have enhanced tumorigenic activity. The basis of this steric effect remains unclear, however, as a result of the decreased tumorigenic activity of the 11-methyl-substituted derivative of 7,12-DMBA. The weak tumor-initiating activity of BA was enhanced at least 4-to 8-fold as a result of methyl-substitution at the 6-and 8-positions (6,8-dimethylbenz[a]anthracene). The higher tumorigenic activity of 6,8-dimethylbenz[a]anthracene compared to BA is consistent with a presumed decrease in metabolic detoxification of the dimethyl-substituted derivative at the 5,6-and 8,9-double bonds.

Metabolism and Mutagenicity of Benzo[c]phenanthrene and its Derivatives. Metabolism of the environmental pollutant and weak carcinogen benzo[c]phenanthrene (B[c]Ph) by rat liver microsomes and by a purified and reconstituted cytochrome P-450 system was examined. B[c]Ph proved to be one of the best polycyclic aromatic hydrocarbon substrates for rat liver microsomes. It is metabolized by microsomes from control rats and from rats treated with phenobarbital or 3-methylcholanthrene at 3.9, 4.2 and 7.8 nmol/nmol cytochrome P-450/min, respectively. Principal metabolites are dihydrodiols along with small amounts (<10%) of phenols. The K-region 5,6-dihydrodiol is the major metabolite and accounts for 77-89% of the total metabolites. The 3,4-dihydrodiol with a bay-region 1,2-double bond is formed in much smaller amounts and accounts for only 6-17% of the total metabolites, the highest percentage being formed by microsomes from control rats. Highly purified monooxygenase systems reconstituted with cytochrome P-450a, P-450b and P-450c and epoxide hydrolase form predominantly the 5,6-dihydrodiol (95-97% of total metabolites) and only a small percentage of the 3,4-dihydrodiol (3-5% of total metabolites). The 3,4-dihydrodiol is formed with higher enantiomeric purity by microsomes from 3-methylcholanthrene-treated rats (88%) than by microsomes from control rats (78%) or phenobarbital-treated rats (60%). In each case the (3R,4R)-enantiomer predominantes. B[c]Ph 5,6-dihydrodiol formed by all three microsomal preparations is nearly racemic.

The mutagenic activities of the enantiomers of the pair of diastereomeric bay-region benzo[c]phenanthrene 3,4-diol-1,2-epoxides were evaluated in histidine-dependent strains of *Salmonella typhimurium* and in an 8-azaguanine-sensitive Chinese hamster cell line. In strains TA 98 and TA 100 of *S. typhimurium*, the range in mutagenic activity observed for the four optically active isomers was less than 4- and 2-fold, respectively. The diol epoxide with (1S,2R,3R,4S) absolute configuration and the benzylic hydroxyl group trans to the epoxide oxygen [(+)-diol epoxide-2] was the most active isomer in both strains. The enantiomeric (-)-diol epoxide-2 isomer, with (1R,2S,3S,4R) absolute configuration identical to that of the exceptionally tumorigenic (+)-diol-epoxide-2 isomers of benzo[a]pyrene, benz[a]anthracene, and chrysene, was the least active isomer in strain TA 98 (27%) and the second most active isomer in strain TA 100 (90%). In Chinese hamster V79 cells (-)-diol epoxide-2 was the most active of the four benzo[c]phenanthrene diol epoxide isomers, and a 4- to 5-fold range in mutagenic activity was observed. The differences in mutagenic activity between the four bay-region diol epoxide isomers of benzo[c]phenanthrene in the three test systems are relatively small when compared with results from similar studies with optically active bay-region diol epoxide isomers of three other polycyclic aromatic hydrocarbons, and may be explicable, in part, by a tendency of the hydroxyl groups of benzo[c]phenanthrene diol epoxides to adopt comparable pseudodiequatorial conformations.

Ovotoxicity of Benzo[a]pyrene Derivatives. Murine ovarian tumors produced by polycyclic aromatic hydrocarbons like benzo[a]pyrene (BP) require small oocyte destruction. Small oocyte destruction was evaluated in C57BL/6N (B6), DBA/2N (D2), and C57BL/6J x DBA/2JF_1 (B6D2F_1) mice following intraovarian injection with BP, (+)-(7R,8S)-oxide, (-)-(7R,8R)-dihydrodiol [(-)-DHD], or (+)-(7R,8S)-diol-(9S,10R)-epoxide-2 [(+)-DE2] at does ranging from 0.01 to 30 µg/ovary. BP, (-)-DHD, and (+)-DE2 produced small oocyte destruction in a dose-dependent fashion. The (+)-(7R,8S)-oxide did not destroy small oocytes at the highest dose tested (10 µg/ovary). The rank orders of the calculated doses which resulted in the destruction of 50% of the small oocytes (ED_{50}s) for small oocyte destruction were BP $\approx$ (-)-DHD > (+)-DE2 in all three groups of mice. However, the ED_{50}s for BP and (-)-DHD differed considerably among B6, D2, B6D2F_1 mice; ED_{50}s were smallest in B6 mice and largest in D2 mice. The ED_{50}s for oocyte destruction in B6D2F_1 mice were intermediate or similar to ED_{50}s for B6 mice, depending on the method used for calculation. In spite of large strain differences in ED_{50}s for BP and (-)-DHD, the ED_{50}s for (+)-DE2 were similar in B6, D2, and B6D2F_1 mice. The similar ED_{50} for (+)-DE2 suggests that it is an ultimate ovotoxin and ovarian carcinogen and that the target molecule(s) and mechanism(s) of detoxification are similar in B6, D2, and B6D2F_1 mice.

Metabolism and Hepatotoxicity of Precocene I. The in vitro metabolism of precocent I by liver microsomes from control and treated rats and the effects of precocene I on the function and histology of the rat liver were examined. The major metabolites (80-90% of total metabolites) from all microsomal preparations were the *cis* and *trans* 3,4-diols of precocene I produced with a *cis/trans* isomer ratio of 1:2. These diols appear to arise mainly by spontaneous hydrolysis of precocene I 3,4-oxide. (+)-(3R,4R)-*cis*- and (-)-(3R,4S)-*trans*-precocene I 3,4-diols were the predominant enantiomers of the 3,4-diol formed. The enantiomeric excess of these diols (2-50%) is dependent on the microsomal preparation, with microsomes from control rats exhibiting the highest stereoselectivity and microsomes from phenobarbital-treated rats the least. 6-Hydroxyprecocene I was the next major metabolite and was formed to the extent of 5% (control), 10% and 17% (phenobarbital and 3-methylcholanthrene treatment, respectively) of total metabolites. Treatment of rats with a single i.p. dose of precocene I (300 mg/kg) resulted in extensive hepatic damage as evidenced by a marked increase of plasma glutamic pyruvic transaminase levels and histologic observation in liver sections of severe centrolobular necrosis. Although phenobarbital treatment of rats increased the rate of liver microsomal metabolism of precocene I by ~50% (nmol products/nmol cytochrome P-450/min) compared to liver microsomes from control rats, hepatic damage caused by precocene I was not significantly affected. Depletion of glutathione levels in the rats with diethyl maleate prior to precocene I treatment dramatically increased the severity of hepatic insult, whereas treatment of the rats with the mixed function oxidase inhibitor piperonyl butoxide prior to treatment with precocene I blocked hepatic damage. Treatment of rats with cysteamine prior to treatment with precocene I protected the animals against the toxic effects. Neither *cis* nor *trans* precocene I 3,4-diol nor 3,4-dihydroprecocene I elicited impaired liver function or cellular damage. The above results are consistent with the view that precocene I 3,4-oxide is the metabolite responsible for the hepatotoxic effects observed when precocene I is injected into rats.

SECTION ON PHARMACODYNAMICS

Pharmacologically Active Compounds from Amphibians and other Natural Sources

Dendrobates arboreus, a new species, was discovered and described from western Panama. Defensive secretions contained 14 piperidine alkaloids including members of the pumiliotoxin-A class and the allopumiliotoxin-A subclass. Remaining alkaloids, including two new compounds are unclassified but appear likely to be indolizidines. Histrionicotoxins were not detected. This new species, one as yet undescribed new species and six other dendrobatid frogs have now been shown to represent a *histrionicus* monophyletic subgroup deserving a generic designation.

Several taxa of small frogs from the Southern Hemisphere contain alkaloids similar or identical to compounds previously known only from Neotropical poison frogs of the family Dendrobatidae. Skin of the Brazilian toad *Melanophryniscus moreirae* (family Bufonidae) contains a new alkaloid 8-hydroxy-8-methyl-6-(5'-hydroxy-2'-methylhexylidene)-1-azabicyclo-[4.3.0]nonane ($C_{16}H_{29}NO_2$), which is designated pumiliotoxin **267C**. Such a structure is typical of the pumiliotoxin-A class of dendrobatid alkaloids. *Melanophryniscus moreirae* contains smaller quantities of an alkaloid ($C_{19}H_{33}NO_3$) identical in chromatographic and mass spectral properties to the dendrobatid alkaloid allopumiliotoxin **323B**. Allopumiliotoxin **323B** and an isomer of **267C** occur with unidentified alkaloids in skin of the Australian frog *Pseudophryne semimarmorata* (family Myobatrachidae), and also in the skin of the Madagascan frog *Mantella aurantiaca* (family Ranidae, subfamily Mantellinae). An extremely potent alkaloid related in structure to pumiliotoxins has been detected from *P. coriacae*. In addition to new compounds, *Mantella aurantiaca* and *M. madagascariensis* also contain other alkaloids (e.g., histrionicotoxin and pumiliotoxin B) that were known previously only in dendrobatid frogs. Such alkaloids have not been detected in a phylogenetically wide array of other anuran amphibians, and the dendrobatid alkaloids thus become an evolutionary enigma. Certain of these compounds may have arisen convergently from new biosynthetic pathways in several families of frogs, or these alkaloids may represent parallel expression of shared-primitive pathways that are unexpressed or lost in related frogs.

Magnetic resonance spectral assignments for carbon-13 and protons of pumiliotoxin A, B and **215D** and of related allopumiliotoxins (7-hydroxy congeners) have been determined. Pumiliotoxin A and its 7-hydroxy congener allopumiliotoxin **323B** can occur as pairs of 15-hydroxy epimers in dendrobatid frogs. Allopumiliotoxin **323B'** and **323B"** and allopumiliotoxin **267A** (the 7-hydroxy congener of **251D**) have 7-axial hydroxy moieties. Allopumiliotoxin **339A** and **339B** are, respectively, the 7-axial-hydroxy and 7-equatorial-hydroxy congeners of pumiliotoxin B. Formation of ring phenyl boronides occurs only with allopumiliotoxin **339B** which is the 7-equatorial hydroxy *cis* to the 8-axial hydroxy of the indolizidine ring.

[^{3}H]Batrachotoxinin-A benzoate ([^{3}H]BTX-B) binds with high affinity to sites on voltage dependent sodium channels in a vesicular preparation from guinea pig cerebral cortex. In this preparation, local anesthetics competitively antagonize the binding of [^{3}H]BTX-B. The potencies of some forty classical local anesthetics and a variety of catecholamine, histamine, serotonin, adenosine, GABA, glycine, acetylcholine and calcium antagonists, tranquilizers, antidepressants, barbiturates, anticonvulsants, steroids, vasodilators, antiinflammatories, anticoagulants, analgesics and other agents have been determined. An excellent correlation with the known local anesthetic activity of many of these agents indicate that antagonism of binding of [^{3}H]BTX-B binding provides a rapid, quantitative and facile method for the screening and investigation of local anesthetic activity. Local anesthetics markedly increase the off rate for binding of [^{3}H]BTX-B.

[^{3}H]Batrachotoxinin-A 20-α-benzoate ([^{3}H]BTX-B) binds specifically and with high affinity (K_d = 30 nM) to a site on voltage-dependent Na^+ channels. Compounds with local anesthetic activity inhibit the binding of [^{3}H]BTX-B by a mutually exclusive, allosteric mechanism. The potential local anesthetic potency of a series of 23 opioids and phencyclidine-like compounds has been estimated by their inhibition of [^{3}H]BTX-B binding to Na^+ channels in a preparation of synaptoneurosomes from guinea pig cerebral cortex. The potency of these compounds were also tested as inhibitors of the specific binding of [^{3}H]phencyclidine ([^{3}H]PCP) to a high affinity site on rat brain membranes. Opioids such as morphine and codeine show little affinity for the [^{3}H]BTX-B binding site or for the [^{3}H]PCP binding site. Other analgesics, many of the PCP-like compounds and dioxadrol derivatives are potent versus [^{3}H]BTX-B binding and display both stereospecificity and high affinity towards the PCP-binding site. However, there was no correlation between local anesthetic potency assessed as antagonism of [^{3}H]BTX-B binding and affinity towards the PCP site. Five classical local anesthetics had no affinity for the PCP-site, but did displace [^{3}H]BTX-B from its binding site.

A series of potentially irreversibly derivatives of local anesthetics have been prepared. The isothiocyanate function was chosen as the alkylating moiety in procaine (Prit) and proparacaine (Proprit) since this group reacts rapidly with amines and thiols, but is essentially inert to hydroxyl groups. This latter property confers stability upon the alkylating ligand in the necessarily aqueous medium for Na^+ channel studies. Both Prit and Proprit inhibit [^{3}H]BTX-B binding irreversibly. Scatchard analysis indicates a typical noncompetitive pattern in the binding of [^{3}H]BTX-B to synaptoneurosomes pretreated with the alkylating ligand. Simultaneous exposure of the preparation to both BTX-B and the alkylating ligand yield competitive plots. The potency of both Prit and Proprit are shifted to the left as compared to the parent compounds. [^{3}H]Prit was prepared by catlytic reduction of dibromoprocaine in the presence of tritium gas, followed by conversion to the isothiocyanate and purification by HPLC. [^{3}H]Prit binds irreversibly. In order to demonstrate the specificity of this binding, synaptoneurosomes were first exposed to non-labelled Prit in the presence of high concentrations of procaine or proparacaine, washed and then exposed to [^{3}H]Prit. Under these conditions, 1.0 to 2.0 picomoles of the ligand were bound per mg protein. Chromatography of this labelled preparation on SDS polyacrylamide followed by fluorography demonstrated the presence of 3 labelled macromolecules with molecular weights of approximately 17,000, 23,000 and 54,000. Reactions carried out in the presence of excess procaine or proparacaine greatly reduced the labelling of the 23,000 and 54,000 dalton macromolecules.

Histrionicotoxin (HTX) at low concentrations of 5-10 μM blocks the postsynaptic potential of the electroplax of *Electrophorus electricus*. At 100-fold higher concentrations, HTX blocks the directly evoked action potentials of the conducting membrane. The pH dependence of the blockade by HTX at synaptic channels is different from that at the conducting membrane. At the synapse HTX is more potent at acid pH, while at the conducting membrane it is more potent at basic pH. HTX at high concentrations antagonizes the effects of batrachotoxin, indicative of an effect on the batrachotoxin-sensitive sodium channels involved in action potential generation. While the effects of HTX on the synaptic channels are concentration, time, and pH dependent, the effects on the channels of the conducting membrane are, in addition, use dependent, suggesting interactions of HTX with the activated forms of these channels.

Histrionicotoxin and twenty-two analogs inhibit binding of $[^3H]$perhydrohistrionicotoxin ($[^3H]H_{12}$-HTX) and of $[^3H]$phencyclidine ($[^3H]$PCP) to sites on the acetylcholine receptor ion complex of *Torpedo* electroplax membranes. Structural alterations to the nitrogen (secondary amine) or oxygen (alcohol) functions or to the five carbon and four carbon side chain of histrionicotoxin alter the potency versus $[^3H]H_{12}$-HTX and $[^3H]$PCP binding measured in the presence or absence of a receptor agonist, carbamylcholine. Histrionicotoxin itself is 3-fold more potent verus $[^3H]$PCP binding than versus $[^3H]H_{12}$-HTX binding. N-Methylation or O-acetylation increases this difference, while alterations to the side chains can either slightly decrease or markedly increase this difference. Histrionicotoxin is some 3.5-fold more potent versus $[^3H]H_{12}$-HTX binding in the presence of carbamylcholine than in its absence. O-Acetylation increases this selectivity for the carbamylcholine-activated state of the receptor channel complex, while alterations in the side chains can either reduce or increase the selectivity. Histrionicotoxin is some 2.2-fold more potent versus $[^3H]$PCP binding in the presence of carbamylcholine than in its absence. N-Methylation of O-acetylhistrionicotoxin greatly increases this selectivity, while alterations in the side chains either reduce or have no effect on selectivity.

Pumiliotoxin B (PTX-B, 6-(6',7'-dihydroxy-2',5'-dimethyl-4'-E-octenylidene)-8-methyl-1-azabicyclo[4.3.0]nonane) increases the force of contractures of spontaneously beating guinea pig atrial strips by 3- to 5-fold with half maximal effects at about 3 μM, and increases rates of atrial contractions by 2- to 3-fold with half maximal effects at about 6 μM. The presence of an axial 7-hydroxy substituent (PTX **339**) decreases the efficacy but not the potency of PTX-B as a positive inotropic agent, while having only slight effects on activity as a positive chronotropic agent. The presence of an equatorial 7-hydroxy substituent (PTX **339B**) greatly decreases efficacy and potency of PTX-B as a positive chronotropic and inotropic agent. Pumiliotoxin A which lacks the side chain 7'-hydroxy group of PTX-B causes only a 2-fold increase in force of contracture at 54 μM , while having minimal effects on rate. The presence of an axial 7-hydroxy substituent (PTX **323B'** and **323B"**, epimeric at the 6'-hydroxy) markedly enhances positive inotropic and chronotropic effects of PTX-A. Another congener, PTX **251D** with a 6-(2'-methylhexylidene) side chain and a synthetic analog with a 6-(6'-heptenylidene) side chain are cardiac depressants. Both lack hydroxyl groups in the side chain. The presence of an ω-1 hydroxy group in the side chain of PTX **251D** yields an alkaloid (**267C**) with weak positive inotropic effects and minimal chronotropic effects. The presence of an axial 7-hydroxy group in the indolizidine ring of PTX **251D** results in a compound (PTX **267A**) with very weak positive inotropic effects, while retaining the negative chronotropic effects of PTX **251D**. A synthetic analog with a 6-(7'-hydroxyheptylidene) side chain is a cardiac depressant even though it contains a side chain hydroxyl corresponding in position to the 7'-hydroxyl of the side chain of PTX-B. The positive chronotropic and inotropic effects of pumiliotoxin B are reversed only by relatively high concentrations of the calcium channel blockers nifedipine and verapamil suggesting that pumiliotoxin B may owe its cardiotonic activities to effects on internal mobilization of calcium.

The novel tricyclic alkaloid gephyrotoxin (GyTX) found in the skin secretions of the frog *Dendrobates histrionicus* potentiated and then blocked the indirectly elicited muscle twitch in a concentration-dependent manner. GyTX prolonged the falling phase of the muscle action potential and decreased delayed rectification supporting the idea that the alkaloid blocks the voltage-sensitive potassium conductance of the electrically excitable membrane. The peak amplitude of the endplate currents and miniature endplate currents were depressed, but no significant deviation from linearity was seen in the current-voltage relationship. The decay time constant of the endplate current was markedly shortened by GyTX. A lack of voltage and time dependence suggests that GyTX has little effect on the acetylcholine (ACh) receptor-ionic channel complex in the closed conformation. At low concentrations GyTX greatly depressed the extrajunctional ACh sensitivity of the chronically denervated soleus muscles. In addition, GyTX produced a strong inhibition of junctional endplate potentials evoked by ACh. Junctional and extrajunctional desensitizations induced by microiontophoretically applied ACh were greatly enhanced by the alkaloid in a frequency-dependent manner. These effects were readily reversible. The interaction of GyTX with binding sites on the acetylcholine receptor-channel (AChR) complex was studied on electroplax membranes from *Torpedo californica*. GyTX binds to the AChR complex at a site distinct from the ACh binding site as revealed by its lack of inhibition of both $[^{125}I]\alpha$-bungarotoxin ($[^{125}I]$BGT) or $[^{3}H]$ACh binding. On the other hand, GyTX at a concentration range between 1 μM and 100 μM significantly increased the potency of the agonist carbamylcholine as an antagonist of binding of $[^{125}I]$BGT. At low micromolar concentrations GyTX inhibited the binding of $[^{3}H]$perhydrohistrionicotoxin and $[^{3}H]$phencyclidine to sites associated with the ionic channel of the AChR complex. The affinity of GyTX for these sites increased 3-5 fold by carbamylcholine. Results of electrophysiological and binding studies indicate that GyTX not only blocks the open channel of the AChR complex but also increases receptor affinity for agonists by enhancing desensitization of the AChR complex.

The actions of the tertiary local anesthetic bupivacaine were studied on nicotinic acetylcholine-ionic channel (AChR) complex using electrophysiological and biochemical methods. Voltage clamp studies of the frog neuromuscular junction revealed a concentration-dependent depression of the decay time constant of the endplate and spontaneous miniature endplate currents. Patch-clamp results indicated that the charged form of bupivacaine blocks the open state of ACh-activated ionic channels interacting with sites at the extracellular segment of AChR complex and creating a species with little or no conductance. Biochemical studies were performed on *Torpedo californica* membrane fragments using [^{3}H]phencyclidine ([^{3}H]PCP) and [^{3}H]perhydrohistrionicotoxin ([^{3}H]H_{12}-HTX) as channel probes. Bupivacaine inhibited the binding of [^{3}H]PCP and [^{3}H]H_{12}-HTX with inhibition constants (Ki) of 32 and 25 µM, respectively. The corresponding inhibition constants for bupivacaine methiodide were 1.8 and 3.2 µM. A preincubation of the membranes with carbamylcholine increased the affinity of bupivacaine for the ionic channel sites 5 to 8 fold and the affinity of bupivacaine methiodide 3 to 4 fold. Bupivacaine, however, had no affinity for the agonist recognition site as determined by [^{3}H]ACh and [^{125}I]α-bungarotoxin bindings. The electrophysiological and biochemical studies indicate that bupivacaine reacts primarily with the ionic channel of the AChR complex. The results are consistent with a sequential model in which the drug interacts with the sites at the ionic channel of AChR complex in its open conformation producing species with little or no conductance. From the present studies there is no evidence for an interaction of bupivacaine with the agonist binding site or closed states of AChR complex.

Pharmacodynamic Amines and Enzymes Involved in Their Metabolism

Catechol-O-methyltransferase. Several areas of research on COMT are being pursued including: 1) Purification and structural analysis of the 23,000 dalton soluble form of this glycoprotein, 2) characterization of multiple forms of COMT including the "membrane-bound" form, 3) further studies on the mechanism of action of COMT especially as pertains to the factors which control the ratio of meta- and para-O-methylation, 4) continued application of specific antibodies to COMT as a tool for the cellular and subcellular localization of the enzyme, 5) the preparation of specific antisera to conjugates of bovine serum albumin with catecholsteroids.

1) An improved procedure for the purification of COMT was developed utilizing affinity chromatogrphy on a column of 3,4-dimethoxy-5-hydroxyphenethylamine agarose conjugate and a 3-cycle gel filtration on Sephadex G100. The protein from this procedure was sufficiently pure to permit analysis of its amino acid composition. It is of interest that this 23,000 dalton form of COMT contains single residues of both cysteine and tryptophan. Carbohydrate analysis of hydrolysates of COMT in methansulfonic acid by HPLC and post column reaction with tetrazolium blue demonstrated the presence of 0.054 moles of mannose per mole of COMT. This latter finding suggests that COMT may be a "high mannose" type of glycoprotein. Our present efforts in this area are directed towards an additional purification step by HPLC of COMT on a rigid, hydrophilic, spherical silica packing. Preliminary results using this technique have yielded a single protein of sufficient purity to initiate studies on the amino acid sequence of COMT.

2) Catechol-O-methyltransferase (COMT) was visualized in homogenates and subcellular fractions of rat tissues, including liver and brain, utilizing gel electrophoresis, electrophoretic transfer of proteins to nitrocellulose ("Western blotting") and immune fixation with antiserum to highly purified, soluble rat liver COMT. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of all tissue homogenates examined revealed three

major immune specific proteins with apparent molecular weights of 23,000, 26,000 and 66,000 daltons (23K, 26K and 66K). Centrifugation of homogenates at 100,000 x g for 60 min enriched the 26K species protein in the pellet; while the 23K and 66K proteins were the predominant forms in the supernatant. The 66K appeared in variable amounts depending on the tissue being examined and the length of transfer of protein and is assumed to be an "aggregate" of the smaller form(s). The 26K protein was essentially the only immunoreactive species seen in a purified preparation of rat liver outer mitochondrial membrane. Isoelectric focusing (IEF) under denaturing conditions and two-dimensional gel electrophoresis of brain and liver fractions showed that the 23K protein resolved into three bands of pI 5.1, and 5.2 and 5.3, while the 26K protein had a pI of 6.2. Analysis of COMT activity in slices from non-denaturing IEF gels indicated that the pI 5.1-5.3 species are biologically active; the pI 6.2 species could not be resolved under these conditions. COMT activity was demonstrated, however, in outer mitochondrial membranes from rat liver which contain predominantly the 26K, pI 6.2 immunoreactive species. The major form of COMT in all rat tissues examined is "soluble" with an apparent MW of 23K and a pI of 5.2. The nature of the modifications giving rise to pI 5.1 and 5.3 forms of this enzyme are not clear, nor is the relationship between the 23K and 26K forms. In order to facilitate the resolution of the multiple forms of COMT, an antisera has been prepared which is directed towards both the major form of COMT (23,000 dalton) and towards the 26,000 dalton form. Work is in progress to utilize selective immunoprecipitation techniques to yield an antisera specific for the 26,000 form of COMT. This form is presumed to be the "membrane-bound" form of COMT.

3) As previously reported antisera to COMT has been used extensively for the cellular and subcellular localization of this enzyme by both immunocytochemical and immunofluorescent methods. Briefly, current studies on COMT localization include:

a) A study of COMT in the rat ovary where COMT is located in the corpus lutea and in ovarian macrophages. The time course of the immunologically defined COMT in uterine macrophages appears just prior to the dissolution of the corpus lutea. Studies of the subcellular localization in macrophages suggest the COMT may be associated with lysosomal enzymes. It should be noted that COMT is a presumptive-high mannose glycoprotein, a structural characteristic of many liposomal enzymes.

b) Examination of the changes in COMT located in the epithelium of the rat uterus has been extended to include an examination of COMT in the entire length of the rat oviduct. Unlike the density of COMT in the uterus no changes in COMT were observed in epithelial COMT extending from the uterotubal junction to the fimbria during the course of pregnancy or the course of estrus/diestreus in non pregnant rats.

c) In a previous study it was shown that COMT appeared in the uterine epithelium at day 4-5, just prior to or coincident with implantation of a fertilized trophoblast. It has now been demonstrated that this sudden increase in epithelial-COMT is not directly related to implantation. Temporal studies in pseudo pregnant rats and rats with induced-delayed implantation display the same sudden increase in epithelial-COMT. This hypothesis is now being investigated.

d) Previous studies on the immunological measurement of uterine epithelial-COMT and uterine metrial gland-COMT during the estrus cycle of non-pregnant rats was limited by the sensitivity of the immunological methodology. Technical improvements, primarily involving elimination of non-specific cytochemical reactions due to endogenous peroxidases, have permitted direct visualization of the changes of epithelial and metrial gland-COMT during the estrus cycle of rat. A detailed examination of these changes is in progress.

e) Previcus studies indicated that catecholsteroids, both 2- and 4-hydroxyestradiol, are substrates with high affinity for COMT. These steroids, derived from estrogen, may play important roles in functions involving reproductive lactation and central mechanisms. In order to investigate these possibilities, antibodies to 2-hydroxyestrone-17-(O-carboxymethyloxine)BSA have been prepared by classical techniques in rabbits. Preliminary immunocytochemical experiments indicate that these antisera can localize catecholsteroids in liver, uterus and hypothalamus. At present the immunological characterization of these antisera, the specificity of the cellular localization, the development of radioimmune assays, and improved techniques of tissue fixation to preserve the localization of catecholsteroids are in progress.

f) Two projects on changes in epithelial-COMT which occur in the uteri of miniature pigs and sheep, utilizing antisera to rat liver COMT, are in progress. In both studies the cross reactivity of the antisera to rat-COMT has been demonstrated. Direct (biochemical) measurement of COMT-activity has been shown to correlate directly with immunological reactivity with antisera to COMT.

g) COMT has been shown to be localized in the endothelium of the aorta, and other large arteries and the virtual absence of COMT in the smooth muscle of arteries. Direct measurements of the O-methylation of d- and ℓ-isoproterenol, incubated with segments of rabbit thoracic aorta in which the intimal surface was removed, suggest that a significant proportion (66%) of the COMT in rat aorta is present in endothelial layer. The anatomical specificity of this localization is under investigation. COMT activity and immunological localization is being measured in cultured cell populations from both smooth muscle, endothelium and fibroblasts.

h) COMT has been shown to be localized in glial cells (astrocytes, oligodendrocytes and microglial cells) in the brains of rat, chinchilla and cow. The occurrence of COMT is now being studied in cultured cell lines derived from human gliomas and astrocytomas. The present evidence has shown cross reactivity between antisera to rat-liver COMT with glial cell cultures derived from human glial tumors.

i) COMT has been shown to be localized in the ductal cells of lactating rat and mouse mammary glands and in adenocarcinomas of rat, mouse and human breast. Current efforts are directed towards an examination of a statistically significant survey of the level of COMT activity and immunocytochemical localization of COMT-reactivity in tissues from human breast tumors and metastatic tumors derived from breast tumors.

Fluorine Substituted Biogenic Amines. Our investigations on the chemistry and biology of ring-fluorinated derivatives of catecholamines, catecholamino acids and related compounds has continued. A unified hypothesis for the mechanism of the fluorine-induced alteration of the biological properties of catechols has been proposed based upon (1) the regioselectivity of the COMT catalyzed O-methylation as well as the relative affinities of the fluorocatechols for this enzyme and (2) the alterations in agonist and affinity for α- and β-adrenergic receptors displayed by fluorocatechols. The kinetic parameters (Km, Vmax, rate constants) of the 2-, 5- and 6-fluoro derivatives of DA and NE, 5- and 6-fluorodihydroxyphenylalanine (6-FDOPA) and 6-F-α-methylDOPA for COMT have been determined. The apparent kinetic constants obtained with these substrates are significantly affected by the site of fluorine substitution. Substitution in the 5-position results in an increase in the affinity of the substrate relative to that of the parent catechol and an increase in the value of Vmax. The turnover rates for 5-FDOPA, 5-FNE and 5-FDA are increased 10, 14 and 20 fold respectively, whereas minimal changes were observed with the 2-fluoro derivatives and with 6-FDA. The apparent Km values for 6-FDOPA, 6-FNE and 6-F-α-methylDOPA were increased 3, 20 and 47 fold with respect to the parent compounds. Confirmation of the low affinity for COMT conferred by fluorine in the 6-position has been obtained from metabolic studies both *in vitro* and *in vivo* where the metabolites via the MAO pathway are readily apparent by greatly reduced or no 6-fluorohomovanilic acid detected. Efforts are in progress to extend this study to include 2-FDOPA, α-methylDOPA and polyfluoro derivatives of the 2,5-, 2,6- and 5,6-difluoro series.

Another characteristic of COMT is its ability to catalyze both meta- and para-O-methylation. Measurements of the pH rate profile for the extent of meta- and para-O-methylation of NE showed that while the absolute amount of meta-O-methylation remained constant from pH 7-10, the extent of para-O-methylation increased with pH with an apparent pKa of 8.5. In order to determine if this increase in para-O-methylation results from increased ionization of the para-hydroxy group of the substrate or a functional group in the active site of COMT a similar study has been carried out with 5-FNE with a phenolic pKa of 7.9. The absolute amount of para-O-methylation of 5-FNE increases with pH to pH 7.9 then decreases, while the amount of meta-O-methylation decreases with pH with a pKa of 7.9. Although these results will require additional interpretation it is clear that the alteration in phenolic ionization alters the extent of meta- and para-O-methylation. This study is being extended to include 2- and 6-FNE and some selected neutral catechols which have previously been shown to yield meta/para ratios of 1.

Previously we reported that the highly selective agonist mediated responses and affinities for adrenergic receptor sites of the 2- and 6-FNE and the 2- and 6-fluoro isoproterenols depended upon the position of fluorine substitution. In order to clarify the apparent α-adrenergic specificity of 6-FNE the 2-, 6- and 4-fluorine derivatives of the specific α-adrenergic agonist, phenylephrine (PE) have been synthesized. Alpha-adrenergic mediated contractions of the guinea pig aortic strip preparation in the presence of cocaine (1 µM) yielded the following potency-rank 6-FPE > (-)PE ≥ 4-FPE >>> 2-FPE. The responses were blocked by phentolamine (1 µM) and unaffected by the presence of propranolol. The affinities of FPE's for the α_1-adrenergic binding site were estimated as antagonists for the specific binding of $[^3H]$prazosin to a rat brain preparation. These results are consistent with our hypothesis that the "fluorine effect" seems to be entirely a "negative" effect, i.e. substitution of fluorine on the 2-carbon significantly decreases the binding of 2-FPE (and 2-FNE) to the α-adrenergic receptor while substitution of fluorine on the 6-carbon significantly decreases the binding of 6-fluoroisoproterenol (and 6-FNE) to the β-adrenergic site. Thus we have proposed that the binding site on the β-adrenergic receptor, like the active site of COMT, contains a positive center directed towards the 6-position on the aromatic ring. Similarly, the binding of NE to the α-adrenergic receptor would involve a binding interaction between a positive center directed towards the 2-position on the aromatic ring. Further, we have proposed that the substitution of fluorine on the 2- or 6-carbon inhibits this interaction by inductive withdrawal of electrons from carbons 2 and 6, resulting in the nearly equal and opposite binding affinities observed with 2- and 6-FNE. Molecular orbitol calculations (MNDO) indicate that fluorine substitution on the aromatic ring of NE reduces the residual electronic charge on the carbon to which it is bound by approximately 0.25 ev.

A study of the chemical properties of the FNE's quickly revealed that they readily undergo oxidation at pH's greater than 7. Initial studies indicated the 5-FNE, 5-FDA formed a highly fluorescent trihydroxyindole derivative when treated with base in a manner similar to NE. However, the 2- and especially the 6-F derivatives failed to form the expected trihydroxyindole. Current studies have shown that 2-, 5-, and 6-monofluoro derivatives of dopamine and norepinephrine show the expected oxidation at pH values equal to or higher than the phenolic pKa. The 6-fluoro derivative appears to be more prone to oxidation than the 2- or 5-fluoro derivatives or the parent catechols. We have studied the effects of fluorine substitution on the oxidation of DA and NE using cyclic voltammetry. The ease of oxidation seen in the NE series, as determined by half-wave potentials was 6-FNE > 5-FNE > 2-FNE > NE, this rank order is consistent with our initial observations. A significant difference was observed in the oxidation of 6-FNE. The oxidation pathway of catecholamines, such as DA and NE, follows a reaction pathway initiated by an electron transfer (oxidation), followed by a chemical oxidation, followed by a second electron transfer. The transfer of 4 electrons can be observed in the oxidation of NE with an appropriate slow scan rate starting with the unprotonated amine. A similar 4 electron pathway was observed for 5-FNE. However, in the oxidation pathway for 6-FNE a maximum of only 2 electrons are transferred. During the oxidation, fluoride ion is released. The oxidation pathway for 2-FNE was less clear apparently following both pathways. The results with the DA series were qualitatively similar.

A continuing series of studies designed to explore the effects of 6-FDOPA and 6-F-α-methylDOPA as well as 6-FDA and 6-FNE on cardiovascular responses are in progress. Infusions of 6-FDOPA in the intact, anesthetized dog yield the expected α-adrenergic receptor mediated responses. Examination of serial blood samples to determine the rate of formation of 6-FDA are in progress. The cardiovascular responses of 2- and 6-F isoproterenol in the intact anesthetized dog to confirm the loss of beta-response by the 6-F derivative seen in isolated organ preparations is in progress. Preliminary studies with 6-F-α-methylDOPA in the intact, unanesthetized SHR rat indicate that like α-methylDOPA it is an effective hypotensive agent. The present results suggest that 6-F-DOPA is a more potent and longer lasting hypotensive agent. This may be a result of the decreased role of O-methylation in the metabolism of 6-F derivatives. Currently we are investigating cardiovascular responses in the rat following the administration of 6-FDOPA, 6-F-α-methyl-DOPA, 6-FDA and 6-FNE into the lateral ventricle, directly into the paraventricular nucleus of the hypothalamus as well as intravenously.

A study on the feasibility of using ^{19}F as an NMR probe to explore the behavior of FNE and FDA's in intact vesicles, whole cells and larger tissue preparations has been accomplished. We have examined the ^{19}F-NMR of 6-FDA in synaptoneurosomes from guinea pig striatum. Both amines are taken up and stored by this preparation in a manner quite similar to the uptake of the parent amines. The ^{19}F-NMR spectra were collected at 4°C. A sharp ^{19}F peak was observed at 37.1 ppm (width 62 Hz) which was identified as 6-fluorodihydroxyphenylacetic acid (6-FDOPAC). A second peak of an unidentified fluorine component was observed at 36.5 ppm (width 65 Hz). In addition, the ^{19}F resonance signal for 6-FDA at 36.5 ppm was present with a line width of 134 Hz. Increasing the temperature to 10°C resulted in the disappearance of the 6-FDA resonance. The greater line width of the 6-FDA signal relative to the cytoplasmic 6-FDOPAC was presumed to result from the restricted motion of 6-FDA sequestered in the intraneuronal storage vesicle.

An ongoing project to utilize the inherent advantages of fluorine as a biological marker has been the development of electron energy-loss spectroscopy (EELS). An efficient method for the detection of light elements has been developed taking advantage of the fact that an electron beam passing through a sample will interact with the K-shell of an element resulting in the loss of a discrete amount of energy corresponding to the binding of the K-shell electron (680 ev for fluorine). Initial studies indicated that mass loss of fluorine due to radiation damage may preclude the use of certain classes of fluorinated analogs, however aryl fluorides withstood doses well within the range necessary to permit fluorine imaging. Our initial attempts at imaging the localization of fluorine as fluorocatechols in various brain preparations (synaptosomes, synaptoneurosomes, etc.) have been unsatisfactory primarily due to difficulties in sample preparation and the heterogeneity of the biological preparations rather than the sensitivity of the fluorine signal. Present efforts are directed towards imaging fluoro derivatives of NE, DA and serotonin following their accumulation by cultures of chromaffin cells. Considerable improvement has been made in sample preparations utilizing a rapid freeze drying technique on carbon grids. Preliminary results suggest that lucent areas 100-300 nM in diameter yielding ^{19}F signals correspond to large storage granules which have accumulated 6-FNE in the chromaffin cell preparation.

The Role of Cyclic Nucleotides in the Nervous System

Forskolin, a cardiotonic diterpene isolated from roots of the Indian herb *Coleus forskohlii* has in the past 2 years been established as a unique and powerful tool for the investigation of both the enzyme adenylate cyclase and the role of cyclic AMP in physiological processes. Forskolin activates adenylate cyclases both in intact cells and in solubilized and particulate membrane preparations. A binding assay for investigation of site(s) involved in the action of forskolin has been developed. Both centrifugation and filtration assays have proven feasible with $[^3H]$forskolin. The binding isotherm in the presence of 5 mM $MgCl_2$ using the centrifugation assay is described best by a two site model: Kd_1 = 15 nM, B_{max1} = 270 fmol/mg protein; Kd_2 = 1.1 µM; B_{max2} = 4.2 pmol/mg protein. Only the high affinity binding sites are detected when the binding is determined using a filtration assay; Kd = 26 nM, B_{max} = 400 fmol/mg protein. Analogs of forskolin that do not activate adenylate cyclase do not compete effectively for $[^3H]$forskolin binding sites. Analogs of forskolin that are less potent than forskolin in activating adenylate cyclase are also less potent in competing for forskolin binding sites. The presence of 5 mM $MgCl_2$ or $MnCl_2$ is required for maximal binding. In the presence of 1 mM EDTA the number of high affinity binding sites is reduced to 110 fmol/mg protein with no change in Kd. There is no effect of $CaCl_2$ (20 mM) or NaCl (100 mM) on the binding. No binding can be detected in membranes from ram sperm which contains an adenylate cyclase which is not activated by forskolin. It is proposed that the high affinity binding sites for forskolin are associated with the activated complex of catalytic subunit and stimulatory guanine nucleotide binding protein.

The effects of forskolin analogs, phosphodiesterase inhibitors and 8-bromo cyclic AMP on plasma exudations induced with bradykinin and prostaglandin E_1 in rat skin were investigated using $[^{125}I]$bovine serum albumin. Forskolin, forskolin 7-ethyl carbonate and 7-desacetylforskolin, which are potent activators of adenylate cyclase, greatly potentiated the bradykinin-induced plasma exudation and inhibited the prostaglandin E_1-induced response. On the other hand, 14,15-dihydroforskolin and 1,9-dideoxyforskolin, which are weak or inactive as activators of adenylate cyclase, did not have any significant effect on bradykinin and prostaglandin E_1-induced plasma exudations. Phosphodiesterase inhibitors potentiated the bradykinin-induced plasma exudation and inhibited the prostaglandin E_1-induced response. 8-Bromo cyclic AMP in the doses of 0.01 to 1 µg potentiated the bradykinin-induced plasma exudation, but had no effect at doses of 10 and 100 µg. 8-Bromo cyclic AMP at all doses significantly inhibited the prostaglandin E_1-induced response. The results suggest that the effects of forskolin and its analogs on plasma exudations induced with bradykinin and prostaglandin E_1 in rat skin derive from activation of cyclic AMP-generating systems. Forskolin also potentiated plasma exudation elicited by thromboxanes.

Adenosine receptors linked to adenylate cyclase have been divided into two classes: An A_1-receptor class is inhibitory to adenylate cyclase while an A_2-receptor class is stimulatory to the enzyme. There appear to be subclasses of A_2-receptors; one subclass has a higher affinity for adenosine analogs than the other. Structure activity relationships for adenosine analogs at the two classes of receptor differ somewhat, while xanthine antagonists show relatively little selectivity for the two classes of receptors. A variety of cyclic AMP-dependent physiological functions appear to be modulated through A_1- or A_2-adenosine receptors. These include inhibition of lipolysis, depression of cardiac function, relaxation of smooth muscle, stimulation of steroidogenesis, enhancement or inhibition of hormone or neurotransmitter release, inhibition of lymphocyte function and platelet aggregation and depression of behavioral activity. Recognition of adenosine by the binding sites on A_1- and A_2-receptors can be thought of as representing the result of interactions at two subdomains, one of which binds the purine moiety and one which binds the ribose moiety. The spatial orientation of these domains is such that apparently only analogs which can exist in a relatively stable anti-configuration can simultaneously bind to both domains and allosterically activate the receptor protein. The ribose recognition domain appears to be of prime importance to activity at adenosine receptors and 2'-hydroxy, 3'-hydroxy and 5'-hydroxy groups contribute strongly to the potency of adenosine analogs as agonists. One modification at the 5'-position results in a potent agonst: That is the replacement of the $-CH_2OH$ group with another moiety capable of hydrogen-bonding, namely the carboxamido group. The highest agonist activity of the adenosine 5'-carboxamides is shown by those with an ethyl-sized nitrogen substituent, either an ethyl group or a cyclopropyl group. These two analogs represent the most potent known A_2-agonists, but still remain more potent as A_1-agonists than as A_2-agonists. The presence of a nonhydrogen donor with relatively high lipophilicity at the 5'-position, as in 5'-methylthioadenosine, results in a potent adenosine antagonist. The affinity of the 5'-methylthioadenosine at A_2-receptors is somewhat greater than adenosine, while at the A_1-receptor, the 5'-methylthio analog is much less potent than the parent compound. The simplest interpretation of the interaction of the ribose moiety of adenosine with its binding subdomain is that hydrogen bond acceptor sites exist which interact with the 2'-OH, 3'-OH and $5'-CH_2OH$ or 5'-CONHR to elicit the allosteric change necessary for activation of the receptor. Purine ring substituents of adenosine can be altered with retention of agonist activity, but the purine ring itself may fit to the purine binding subdomain in a very exact manner. Most ring-altered adenosines have no or minimal activity at adenosine receptors. These include 7-deazaadenosine, 8-azaadenosine, 8-aza-9-deazaadenosine, 3-deazaadenosine, and 7-deaza-8-azaadenosine. A 2-fluoro or 2-chloro substituent reduces activity slightly, while 2-hydroxy and 2-amino substituents cause significant reductions at adenosine receptors. 8-Substituents are not tolerated by adenosine receptors in direct relationship to their destabilization of the requisite anti-configuration of the adenine and ribose moieties. The presence of an amino group at the 6-position is essential to potent activity as an adenosine agonist. The simplest interpretation is that a relatively basic amino-moiety at the 6-position is essential to interact with a hydrogen bond donor of the purine subdomain and thereby elicit the allosteric change necessary for the activation of the receptor.

The effect of N^6-substituents on activity of adenosine analogs is currently being studied in collaboration with Dr. R. Olsson and colleagues (U. South Florida, Tampa) with regard to the topography of the N^6-substituent subdomain of A_1- and A_2-receptors. An N^6-methyl substituent reduces potency at A_1- and A_2-receptors by about 10 fold. This suggests that steric or other factors mitigate against the presence of a methyl group on the N^6-nitrogen. Two N^6-alkyl substituents as in N^6-dimethyladenosine nearly completely eliminate activity at A_1- and A_2-receptors. Surprisingly, increasing the N^6-alkyl substituent from methyl to ethyl does not further decrease potency. Instead it causes a reversal in activity and N^6-ethyladenosine is many fold more potent than N^6-methyladenosine at both A_1- and A_2-receptors. The simplest explanation is that interaction of the second carbon with a hydrophobic site alters either the steric or other factors that had mitigated against the first carbon. The situation at the N^6-substituent domain is, however, complex and the topography and ability to change to accomodate changes in the N^6-substituent at A_1- and A_2-receptors requires a detailed study. N^6-Phenyladenosine and N^6-phenethyladenosine are quite potent as adenosine receptor agonists, while the middle member of this series of three analogs, namely N^6-benzyladenosine, has many fold lower activity at both A_1-and A_2-receptors. Stereoselectivity of the N^6-substituent binding domain of A_1- and A_2-receptors differs significantly. The N^6-**R**-phenylisopropyladenosine (**R**-PIA) is much more potent at A_1-receptors than the diastereomeric N^6-**S**-phenylisopropyladenosine (**S**-PIA). The stereoselectivity at A_1-receptors ranges from 10-200 fold. However this pair of diastereomers also exhibits stereoselectivity at A_2-receptors where the **R**-isomer is from 3-14 fold more potent than the **S**-isomer. Thus, both receptors have stereoselective N^6-substituent binding subdomains which differ in their degree of stereoselectivity. It seems most likely that the initial fit of either the $C_6H_5CH_2$- or CH_3-moiety into this subdomain places the other substituent into a sterically or electronically less favorable environment in the case of the **S**-isomer. Other pairs of diastereomeric N^6-substituted adenosine exhibit greater or lesser selectivity at A_1- and A_2-adenosine receptors and may prove more useful probes than **R**- and **S**-PIA.

Theophylline and caffeine, prototypes for adenosine-receptor antagonists of the xanthine class, are virtually non-selective for A_1- and A_2-receptors. It is, therefore, unclear to what extent the antiasthmatic, diuretic, respiratory stimulant, central stimulant, antilipolytic, hypothermic, cardiac stimulant, and analgesic adjuvant activities of xanthines reflect interactions at A_1- or A_2-receptors or some other site. An 8-phenyl group markedly enhances the potency of theophylline at adenosine receptors, but does not confer selectivity. A series of 8-(aryl substituted)phenyl derivatives of theophylline and other 1,3-dialkyl xanthines have been evaluated for potency and selectivity as antagonists at A_1- and A_2-adenosine receptors in brain tissue. Theophylline has a similar potency (K_i 14 μM) at both A_1- and A_2-receptors. 8-Phenyltheophylline is 25-35 fold more potent as an adenosine receptor antagonist than theophylline, while 8-phenylcaffeine is only 2-3 fold more potent than caffeine. A p-hydroxy aryl substituent enhances the potency of 8-phenyltheophylline as an adenosine antagonist. p-Carboxy and p-sulfo aryl substituents reduce potency of 8-phenyltheophylline yielding water soluble adenosine antagonists, which are some 2 to 5 fold more potent than theophylline at adenosine receptors. None of the 8-(aryl substituted)phenyl theophyllines are particularly selective as antagonists towards A_1- and A_2-adenosine receptors. 1,3-Dipropyl-8-phenylxanthine represents a potent and somewhat selective A_1-receptor antagonist about 23 fold more potent at A_1-receptors than at A_2-receptors. A p-hydroxy aryl substituent further enhances potency of the 1,3-dipropyl-8-phenylxanthine at both A_1- and A_2-receptors. The 8-(2-amino-4-chlorophenyl)-1,3-dipropylxanthine is a very potent and selective antagonist for A_1-receptors being nearly 400 fold more potent at A_1- than at A_2-receptors. The water soluble 8-(p-sulfophenyl)- and 8-(p-carboxyphenyl)-1,3-propylxanthines no longer exhibit marked selectivity. Both compounds are much more potent as adenosine antagonists than theophylline. The striking selectivity of 1-isoamyl-3-isoamylxanthine as an A_1-antagonist is retained in the 8-phenyl derivative but is virtually lost in the 8-p-sulfophenyl derivative.

A variety of mesoionic xanthines including mesoionic thiazolo[3,2-α]pyrimidines, benzothiazolopyrimidines, and 1,3,4-thiadiazolo[3,2-α]pyrimidines were antagonists of A_1-adenosine receptors (inhibition of binding of [^{3}H]cyclohexyladenosine) and A_2-adenosine receptors (inhibition of 2-chloroadenosine-elicited accumulations of cyclic AMP) in brain tissue. Most of the compounds were less potent than theophylline and none were remarkably selective for A_1- or A_2-adenosine receptors. However, members of the thiadiazolopyrimidine class of mesoionics exhibited very low or no activity as antagonists of A_2-adenosine receptors while exhibiting activity only 2-4 fold lower than that of theophylline at A_1-adenosine receptors. Unlike the case for theophylline, the presence of phenyl substituent in the 5-membered ring did not enhance the potency of a mesoionic thiadiazolopyrimidine. The nature of the substituents on the mesoionic ring did not appear to have marked effects on potency unlike the marked effect of the nature of 1,3-substituents on activity of non-mesoionic xanthines. The benzothiazolo[3,2-α]pyrimidines were the most potent antagonists being nearly as potent as theophylline at A_1-adenosine receptors and somewhat more potent than theophylline at A_2-adenosine receptors. Other heterocycles with significant adenosine antagonist activity included 1,3-dimethyl-7-phenyllumazine, a pteridine analog of 8-phenyltheophylline, etonitazene and its parent 1-methyl-2-phenylbenzimidazole, chlordiazepoxide, prazocin, ethyl-β-carboline-3-carboxylate, the pyrazolopyrimidines cartazolate, etazolate and tracazolate, and certain "8"-substituted pyrazolo- and imidazopyridines and carbamazepine. Etazolate, an anxiolytic agent, exhibits relatively high potency at A_1-receptors, being 60 fold more potent at A_1- than at A_2-receptors. Modifications of its structure based on analogies to the xanthines have only yielded relatively inactive congeners.

The anticonvulsant carbamezepine inhibits binding of 1 nM [^{3}H]N^6-cyclohexyladenosine to rat cerebral and cerebellar A_1-adenosine receptors with an IC_{50} value of about 50 μM. This concentration is well within that expected for therapeutic regimens. Other anticonvulsants such as phenobarbital, phenytoin, primidone, valproate sodium, and ethosuximide had little or no effect on binding, while theophylline and caffeine caused marked inhibition. Carbamazepine had no marked effect on cyclic AMP levels in guinea pig cerebral cortical or hippocampal slices, but was a weak inhibitor (IC_{50} about 200 μM) of 2-chloroadenosine-elicited accumulations of cyclic AMP *via* an A_2-adenosine receptor in cortical slices. Carbamezepine is thus, a somewhat selective ligand for A_1-adenosine receptors in brain. The nature of its activity at those receptors is unclear, but its lack of central stimulant effects contrasts to the stimulant properties of A_1-adenosine receptor antagonists such as caffeine and theophylline.

Adenosine and various analogs potentiated plasma exudation elicited by bradykinin in rat skin using $[^{125}I]$-labelled bovine serum albumin ($[^{125}I]$-BSA) as a tracer. L-N^6-Phenylisopropyladenosine (L-PIA) was much more effective than D-PIA, adenosine, N^6-cyclohexyladenosine (CHA) and 2-chloroadenosine, all of which were comparable in activity. Adenosine 5'-cyclopropylcarboxamide was the least effective analog. Caffeine and theophylline had no effect on basal or bradykinin-elicited plasma exudation, while inhibiting plasma exudation elicited by L-PIA, CHA or a combination of bradykinin and L-PIA. 8-Phenyltheophylline was more potent than caffeine or theophylline versus the bradykinin and L-PIA combination. 2'-5'-Dideoxyadenosine, a P-site inhibitor of adenylate cyclase, had no effect on plasma exudation elicited by bradykinin, L-PIA or a combination of bradykinin and L-PIA, but did inhibit plasma exudation elicited by prostaglandin E_1 (PGE_1) or a bradykinin-PGE_1-combination. The antihistamine cyproheptadine slightly reduced plasma exudation elicited by a bradykinin-PGE_1 combination. The results suggest that adenosine potentiates bradykinin-induced plasma exudation *via* an A_1-adenosine receptor and that histamine may be involved to some extent in the phenomenon.

A particulate preparation obtained by low speed centifugation of guinea pig cerebral cortical homogenates prepared with a Krebs-Ringer glucose bicarbonate (KRGB) buffer contains cyclic AMP-generating systems responsive to various neurotransmitters. Light microscopic examination, using a birefringence detection system, reveals the presence of intact neurons, axonal fragments, glial cells and erythrocytes along with an abundance of spherical vesicles (1.1 μM) to which one or more smaller vesicular entities (0.6 μM) are attached. The latter are postulated to be synaptosomes while the former would be the attached resealed postsynaptic entity. Filtration of initial KRGB homogenates through 5 or 10 micron Millipore filters followed by low speed centrifugation yields a particulate preparation composed mainly of these synaptosomes with an attached postsynaptic entity: These will be referred to as synaptoneurosomes. Filtration reduces levels of markers for whole cells and endothelial cells while neuronal markers such as acetylcholinesterase activity and norepinephrine uptake are increased. There is no apparent change in the density of a range of central nervous system receptors or ion channels. Levels of A_1-adenosine and H_1-histamine receptors are increased, while levels of so-called peripheral benzodiazepine-binding sites are decreased. Levels of S-100 protein, a marker for glial cells, are not significantly decreased. Accumulations of cyclic AMP elicited by 2-chloroadenosine, histamine, norepinephrine and combinations of these agents are lower in filtered than in unfiltered preparations. There is a decrease in basal adenylate cyclase activity after filtration but no change in phosphodiesterase activity. A phosphodiesterase inhibitor has no effect on accumulations of cyclic AMP in filtered preparations suggesting that regeneration of ATP may have become rate-limiting with respect to cyclic AMP-generation. Carbamylcholine, norepinephrine, and histamine increase formation of inositol phosphate in brain slices. Responses are markedly reduced in synaptoneurosome preparations, apparently in parallel with the ability of norepinephrine and histamine to cause synergistic accumulations of cyclic AMP.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 31100-19 LBC

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pharmacologically Active Compounds from Amphibians and Other Natural Sources

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Some two hundred pharmacologically active alkaloids have now been identified in extracts of frog skins. The batrachotoxins (steroidal) are potent activators of voltage-dependent sodium channels. Antagonism of binding of radioactive batrachotoxinin-A benzoate to brain synaptoneurosomes provies a rapid assay for local anesthetic activity of a wide range of drugs. Histrionicotoxins (spiropiperidines) block voltage dependent sodium and potassium channels and the nicotinic receptor-channel complex. Structure activity for natural and synthetic histrionicotoxins at the nicotinic receptor-channel complex has been assessed using binding of radioactive perhydrohistrionicotoxin as a probe. Pumiliotoxins (6-alkylidene-8-hydroxy-8-methylindolizidines) and allopumiliotoxins (7-hydroxypumiliotoxins) enhance evoked release of acetylcholine and augment direct or indirect-evoked muscle contraction in neuromuscular preparations and have positive chronotropic and inotropic effects in heart. These effects are markedly dependent on structure and appear due to alkaloid-induced augmentation of release of calcium from intracellular storage sites. Gephyrotoxins (perhydrobenzoindolizidines), like the histrionicotoxins, block the nicotinic receptor-channel complex. Gephyrotoxin appears to not only block the open channel but to accelerate desensitization of the receptor channel complex. Certain local anesthetics, such as bupivacaine, also block the nicotinic receptor-channel complex through interaction at histrionicotoxin-sensitive sites.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 31101-16 LBC

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Pharmacodynamic Amines and Enzymes Involved in Their Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	C.R. Creveling	Research Chemist	LBC, NIADDK
Others:	J.W. Daly	Chief	LBC, NIADDK
	E.T. McNeal	Biologist	LBC, NIADDK
	G. Lewandowski	Guest Worker	LBC, NIADDK

COOPERATING UNITS *(if any)*

B. Hartman, Washington, U., St. Louis, MO; R.T. Borchardt, R. Adams, M. Rice, U. Kansas, Lawrence, KA; L.W. Tice, LEP, NIADDK; M. Lowe, NHLBI; K.L. Kirk, L.A. Cohen, A. Jacobson, T. Burke, LC, NIADDK; R.M. Weinshilboum, Mayo Clinic,

LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Section on Pharmacodynamics

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.9	0.6	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type Do not exceed the space provided.)*

The chemistry, biochemistry, physiology, and pharmacology of biogenic amines, their amino acid precursors and metabolic products, and various synthetic derivatives have been investigated. The areas of specific interest are: 1) The immunohistochemical and immunofluorescence localization of catechol-O-methyltransferase (COMT) in malignant, physiologically and hormonally modified, and normal tissues of rodent, porcine, and human at the light and electron microscopic level. 2) The examination of the temporal and hormonal relationship between uterine epithelial COMT during the course of pregnancy in rat and pig. 3) The relationship betwen breast ductal epithelial-COMT and lactation in rat and mouse. 4) The localization and level of COMT in both normal and malignant breast tissues in woman, the relationship of COMT between both type and extent of malignancy, and the occurance of increased COMT levels in gynomasty in man. 5) The immunolocalization and measurement of 2-hydroxyestradiol in uterus, liver and hypothalamus of rat. 6) The determination of the amino acid sequence and the structure of the polysaccharide component of the 23,000 dalton form of COMT from the rat liver. 7) The effect of fluorine substituents on the aromatic ring of catecholamines, tyramines, 5-hydroxyindoleamines, and their amino acid precursors on the physical properties and on the interaction with alpha and beta receptor systems, the active site of COMT and monoamine oxidases. 8) The uptake and metabolism of 6-fluoronorepinephrine and 6-fluorodopamine in preparations from rodent brain measured by HPLC and by F-18 NMR spectroscopy. 9) The development of systems for the use of F-19 positron tomography to image the distribution of fluorodihydroxyphenyalanine in the brains of intact rodents. 10) The mechanism of toxicity of 2-fluorohistidine in normal and leukemic mice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 31102-13 LBC

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Role of Cyclic Nucleotides in the Nervous System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.W. Daly	Chief	LBC, NIADDK
Others:	C.R. Creveling	Research Chemist	LBC, NIADDK
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	W. Padgett	Biologist	LBC, NIADDK
	K. Seamon	Guest Worker	FDA
	M. Shamim	Guest Worker	LBC, NIADDK
	E.T. McNeal	Biologist	LBC, NIADDK

COOPERATING UNITS *(if any)*
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LAB/BRANCH
Laboratory of Bioorganic Chemistry

SECTION
Section on Pharmacodynamics

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	1.6	1.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Forskolin, a cardiotonic diterpene, activates adenylate cyclase in solubilized and particulate preparations and in intact cells: A binding assay with radioactive forskolin provides a means of investigation of the site of action of this diterpene and possible interactions with hormones, drugs, and endogenous factors with forskolin binding sites. Forskolin analogs potentiate plasma exudation elicited by bradykinin, histamine and thromboxanes, but block exudations elicited by prostaglandins and adenosine analogs. Adenosine analogs potentiate exudations elicited by bradykinin and the effect is blocked by caffeine and other xanthines. Structure activity relationships for nearly one hundred and fifty adenosine analogs at A1- and A2-adenosine receptors have been delineated using binding assays and physiological responses. Certain analogs with specificity for A1-adenosine receptors have been developed. A variety of xanthines have been prepared and investigated as antagonists of A1- and A2-adenosine receptors. Certain 8-phenyl-xanthines with 1,3-dipropyl substituents are both very potent and somewhat selective antagonists at A1-receptors. Polar substituents on the 8-phenyl moiety confer desirable water solubility, but reduce both potency and selectivity. Other heterocyclic compounds exhibit antagonist activity at adenosine receptors, but most are not particularly potent or selective. These include carbamazepine, benzodiazepines, pteridines, mesoionic thiazolopyrimidines, thiadiazolopyrimidines and benzothiazolopyrimidines, pyrazolopyrimidines and pyrazolopyridines. A facile filtration technique yields a brain synaptoneurosome preparation which can be used for investigation of interrelationships of receptor and channels, cyclic AMP-generation, phospholipid turnover, membrane potential, and ion fluxes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 31103-06 LBC

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Receptors for Neurotransmitters and Drugs in the Central Nervous System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

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	G.T. Bolger	Visiting Fellow	LBC, NIADDK
	B.A. Weissman	Guest Worker	LBC, NIADDK
	H. Lueddens	Guest Worker	LBC, NIADDK
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COOPERATING UNITS *(if any)*

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LAB/BRANCH

Laboratory of Bioorganic Chemistry

SECTION

Section on Neurobiology

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
6.1	5.5	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

High affinity, stereospecific recognition sites (receptors) for putative neurotransmitters, neuromodulators, and many psychotherapeutic agents have been identified in the mammalian central nervous system. It is currently thought that the interaction of a neurotransmitter, neuromodulator, or psychotherapeutic agent with these sites initiates a series of events resulting in either a physiologic/behavioral response (in the case of neurotransmitters and neuromodulators) or a pharmacologic action (in the case of a psychotherapeutic agent). The high affinity, stereoselective binding of psychotherapeutic agents to brain also suggests that previously undescribed endogenous modulators which physiologically mimic (or antagonize) the actions of these agents may also be present. Several receptor systems are currently under study, including: a) "Brain-type" benzodiazepine receptors, believed to play a role in the pathophysiology of anxiety, seizure disorders, musculoskeletal disorders, and sleep disorders. These receptors are part of a "supramolecular complex" which contains recognition sites for benzodiazepines, gamma-aminobutyric acid, and a chloride ionophore. b) The "peripheral-type" recognition sites for benzodiazepines that are found in both central and peripheral tissues. c) Receptors for central stimulants (e.g., amphetamine). d) Receptors for hallucinogens (e.g. phencyclidine), and e) Recognition sites for drugs which regulate calcium flux.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 31104-16 LBC

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Enzymatic Oxidation of Drugs to Toxic and Carcinogenic Metabolites

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Donald M. Jerina, Chief, Section on Oxidation Mechanisms, LBC, NIADDK

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COOPERATING UNITS *(if any)*

Dept. of Experimental Carcinogenesis and Metabolism, Hoffmann-La Roche (Nutley, NJ); Dept. of Chemistry, U. Maryland (Catonsville); Dept. of Chemistry, U. Oklahoma (Norman); and the Queen's U. of Belfast (N. Ireland)

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Laboratory of Bioorganic Chemistry

SECTION

Section on Oxidation Mechanisms

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
9	8	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The primary goal has been the elucidation of the structure of reactive metabolites which are responsible for the carcinogenic, cytotoxic, and mutagenic activity of benzo[a]pyrene and other polycyclic hydrocarbons. The approach taken consists of: i) synthesis of primary oxidative metabolites as well as selected secondary oxidative metabolites, ii) study of the metabolism of these hydrocarbons with liver microsomes, as well as with purified and reconstituted cytochrome P-450 systems with and without epoxide hydrolase, iii) tests for inherent mutagenicity of the synthetic metabolites toward bacterial and mammalian cells, iv) elucidation of the roles of the cytochrome P-450 system and epoxide hydrolase in potentiating or obliterating the mutagenicity of these metabolites, v) determination of the carcinogenic activity of these compounds, vi) determination of the rate of formation and nature of the products formed when reactive metabolites such as arene oxides and diol epoxides react with biopolymers and less complex model compounds, and vii) search for compounds capable of preventing the tumorigenic action of bay-region diol epoxides. A general theory of hydrocarbon-induced carcinogenesis, the bay-region theory, has been formulated and its predictions are being tested with synthetic potential metabolites of several hydrocarbons. Based on the presently available data, the bay-region theory has excellent predictive value. Comparative solvolytic studies of benzo-ring diol epoxides in which either the diol portion or the epoxide portion forms part of a bay region have established that preferred conformation is a major factor in determining reaction rate. Tumorigenicity is not predictable based on solvolytic reactivity alone. Biological studies have shown that a bay-region 3,4-diol-1,2-epoxide of benz[c]acridine is an ultimate carcinogen. The bay-region (+)-(1R,2S,3S,4R) diol epoxide of benz[a]anthracene has been established as the most important ultimate carcinogen of this hydrocarbon. Several naturally occurring plant phenols have been shown to have potent antimutagenic activity toward bay-region diol epoxides. A new synthesis of optically active K-region arene oxides has been devised.

Annual Report of the Laboratory of Molecular Biology

National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases

The Laboratory of Molecular Biology has as its principal goal the understanding of biological processes at the molecular level. The research program involves application of both theoretical and experimental methods to a wide variety of problems in molecular genetics, regulation of gene expression in eukaryotes, mechanisms of DNA replication, nucleic acid and protein structure, bioenergetics and transport properties. Specific areas under investigation include the structures and chemical properties of biologically important materials. These involve studies of the organization of DNA and proteins within the eukaryotic nucleus, investigations of enzyme and immunoglobulin structures by x-ray diffraction, investigation of polynucleotide chemistry and structure by synthetic and spectroscopic methods, studies of polynucleotide structure by fiber diffraction methods and of protein interactions by calorimetric methods, studies of the conformations of supercoiled DNA and their effect on biological properties, and theoretical studies of the mechanism of energy conversion in biology, muscle contraction, mictrotubule formation, ion transport and biochemical kinetics. Other investigations are concerned directly with biological processes. These include studies of the process of transformation by the tumor virus SV40, and of the mechanisms of genetic recombination and DNA replication. Important advances have been made in these areas this year.

Chromatin Structure and Function

We have continued our studies of chromatin structure, in order to understand the forces that are involved in the packaging of DNA within eukaryotic nuclei and to determine how that structure is modified in the neighborhood of genes tha are being expressed. We have extended studies of the phasing of nucleosomes on DNA, and have found that for at least one arbitrarily chosen 145-base-pair DNA fragment, the placement of the histone octamer with respect to the ends of the DNA is asymmetric, showing that there are strong sequence-dependent forces of interaction between protein and DNA. In experiments concerned with the structure of chromatin containing transcriptionally active genes, we have examined the physical properties of nucleosome-like particles containing the chicken adult β-globin gene, isolated from erythrocytes in which that gene is being expressed. We find that these particles possess properties very similar to those of normal nucleosome core particles, showing that the packaging of active genes at this level of organization is similar to that of bulk chromatin. We have also continued studies of the structure of DNA in the promoter region of this β-globin gene, which we have found has a special chromatin structure when the gene is expressed, as demonstrated by an unusual sensitivity to nucleases. We have been able to reconstruct this sensitive structure by assembling chromatin on globin-gene containing plasmids in the presence of extracts from chicken erythrocyte nuclei. We have shown that these extracts contain one or more factors that bind specifically and tightly to the promoter region, and that appear to be responsible for generating the unusual chromatin structure of the region. The factor has been partially purified.

Three-Dimensional Structure of Proteins of the Immune System

A significant advance has been made in our efforts to study, by X-ray diffraction, antibody-antigen complexes. The small crystals previously obtained of the Hy10-FAB-lysozyme complex have been increased in size by the application of microseeding techniques to the point where they could be used for X-ray diffraction studies. X-ray film data have been collected at the Cornell High Energy Synchrotron Source and are currently being processed.

The three-dimensional structure of McPC603, a mouse anti-phosphocholine myeloma Fab, has been completely refined. The refined structure has good stereochemistry and the refined coordinates have been used in an analysis of the mode of binding of phosphocholine.

The three-dimensional structure of J539, an anti β (1,6)-D-Galactan mouse myeloma Fab has been refined. The model is being examined to try and locate the site of galactan binding.

Three-Dimensional Structure of Enzymes

A comprehensive modeling study has been undertaken of renin, an enzyme responsible for the cleavage of angiotensinogen to angiotensin I, and therefore a key enzyme in the control of hypertension. The model has been based on the structures of homologous fungal proteases and control experiments indicate that when reasonable sequence homology prevails, the model will closely match the actual structure. These models can be used in the absence of a crystal structure to model inhibitors for the enzymes.

Crystals of tryptophan synthetase from *Salmonella typhimurium* have been examined and shown to be very suitable for a high resolution X-ray analysis.

Neutron diffraction studies have been used in a high resolution study of hydrogen exchange in bovine pancreatic trypsin inhibitor. The results are in excellent agreement with the results obtained by an NMR study.

$2NH_2A{\cdot}T$ Helices in the Ribo and Deoxypolynucleotide Series

Polynucleotide helices formed by the interaction of $(d2NH_2A)_n$, $(r2NH_2A)_n$, $(dT)_n$, and $(rT)_n$ have been prepared and their physical and spectroscopic properties examined. Thermal transitions, dependence of T_m on salt concentration, stoichiometry, phase diagrams, calculated enthalpies, UV, CD, and IR spectra have been determined. All of the deoxy-deoxy helices containing $2NH_2A$ have positive CD first extrema near 290 nm and appear to have B-form structure. All the ribo-ribo or hybrid helices have negative first extrema in this region and appear to have A-form structure. Elevation of T_m and ΔH by the $2NH_2$ group of $2NH_2A$ is much smaller in the deoxy than in the ribo series. We attribute these effects on T_m and ΔH in the deoxy series to loss of stabilization of the spine of hydration in B-form structures caused by interference of the $2NH_2$ group in the minor groove of the helix.

Synthetic Deoxyoligonucleotides

We have synthesized several defined sequence deoxyoligonucleotides by a solid phase phosphotriester procedure for investigation by physical and biochemical methods. We find that GGATCCGGATCC serves as a Template-primer for DNA polymerase I, though previous workers report that oligomers containing more than 5 residues in the repeat do not serve as templates. We have determined the salt dependence curve and dependence of T_m of the oligomer on concentration. NMR investigation of this oligomer is now in progress as part of a collaborative project with Dr. Girjesh Govil of the Tata Institute for Fundamental Research in Bombay, India.

Infrared Spectroscopy

By using ZnSe cells and subtracting solvent spectrum by computer, we can observe bands at 812 and ~ 865 cm^{-1}, characteristic of A-form structures, and bands at ~ 840 and 970 cm^{-1}, characteristic of B-form structures. Preliminary results suggest that some but not all RNA-DNA hybrids have different conformations in the two strands.

Thermal Measurements of Biomolecular Systems

(A) Phospholipid Membranes

Using dipalmitoylphosphatidylcholine liposomes as a model for the cell membrane, calorimetric measurements have shown that both anesthetic and non-anesthetic steroids depress and broaden the gel to liquid crystalline phase transition of the bilayer but there is no correlation with anesthetic potency. An excellent correlation was found, however, between anesthetic potency and the more subtle but less well understood pretransition of the gel phase. Raman spectroscopic measurements demonstrate that the steroid molecule binds within the acyl region of the bilayer. These results give support to hypotheses relating anesthesia to lipid perturbation.

(B) Nucleic Acids

DNA - The B → Z transition of d5MeC-dG at moderate salt concentration has been found to take place with a vanishingly small enthalpy change demonstrating that this process is entropically driven.

RNA - The thermodynamics of melting to single strands of helical duplexes formed between poly(rU) and homopolymers of poly(rA), poly(r2NH_2A) and several random poly(2NH_2,A) copolymers have been studied by differential scanning calorimetry. the enthalpies of transition, ΔH, are 8.4 kcal/2P for poly(rA)·poly(rU) melting at 56°C and 14.7 kcal/2P for poly(r2NH_2A)·poly(rU) melting at 89°C. The large difference in the value of ΔH for these two homopolymer complexes reflects the profound contribution of the 2-amino group to the energetics of base pairing. At 90°C, where there is little or no self-structure of the single strands of poly(rA) or poly(r2NH_2A), the data shows the r2NH_2A•rU base pair to be about 4.5 kcal/2P were stable in ΔH than the rA•rU pair.

Influences of Macromolecular Crowding on Biochemical Systems

To our initial studies of the effects of macromolecular crowding on enzymatic DNA joining, we noted that T4 DNA ligase could be enormously stimulated by high concentrations of polymers. We here extend those studies to a variety of DNA substrates.

The rates of blunt-end and cohesive-end ligation of DNA by T4 DNA ligase are increased by orders of magnitude in the presence of high concentrations of a variety of nonspecific polymers such as polyethylene glycol, Ficoll, bovine plasma albumin, or glycogen. Blunt-end ligation of small self-complementary oligodeoxyribonucleotides is also stimulated. The use of polyethylene glycol 6000 in such systems is characterized in some detail. Conditions are suggested which either greatly increase the rate of formation of and size of linear ligation products or which allow smaller but significant improvements in the amounts of circular ligation products.

A patent covering these results on polymer-stimulated enzymatic ligation of DNA was applied for on behalf of the government.

Genetics and Structure of the Oncogenic Virus, SV40

We have isolated a number of clones believed to contain the origins of DNA replication in monkey cells. A detailed analysis of nine of these has been completed. Only two contain unique sequences. The remaining seven contain moderately reiterated or highly reiterated sequences. One moderately reiterated monkey sequence of 196 bp (base pair) has been found in two different clones. All of the sequences contain extensive AT-rich regions and potential stem-loop structures. Six of the nine share between 13 and 19 bp of a 21 bp "consensus sequence." Four of five tested replicate preferentially early in S phase. None has been shown to act as a plasmid replication origin.

In other experiments, we have generated deletions and substitutions of the 27 bp perfect inverted repeat sequence (13x1x13) at the SV40 replication origin.

A substitution mutant, in which an entirely different 13x13 perfect repeat has been substituted for the original 13x1x13 sequence, is incapable of binding the SV40 T antigen. Heteroduplex molecules between the wild type and mutant molecule obligatorily exist as cruciform structures, and on reconstitution with histones cause nucleosome phasing.

Replication of ColE1 DNA

Studies on DNA replication of plasmid ColE1 and its relatives have been continued. Transcripts by RNA polymerase (RNA II) that start the 555 nucleotides upstream of the replication origin of plasmid ColE1 extend beyond the origin. The hybridized transcript is cleaved by ribonuclease H at the origin and used as the primer for DNA synthesis by DNA polymerase I.

The primer formation is dependent on the secondary structure of RNA II. A single base change far from the replication origin can affect a specific stage of primer formation. Thus each single base-change affects initiation of synthesis of RNA II, hybrid formation between RNA II and the template DNA, the cleavage sites of RNA II by RNase H, the stability of association of RNA II or the use of cleaved RNA II as a primer by DNA polymerase I. Suppressor mutations that restore the activity of RNA II lost by a mutation have been found. Based on the results of analysis of mutant RNA IIs by partial digestion with an RNase we could deduce the structure of functional RNA II.

Primer formation is regulated by a plasmid-specified small RNA (RNA I). RNA I binds to RNA II at the complementary region. This binding results in inhibition of formation of the secondary structure necessary for primer formation. Based on the results of kinetic analysis of binding of RNA I to RNA II, we proposed a stepwise model of binding of RNA I to RNA II.

Primer formation is also regulated by a protein specified from a regio downstream of the replication origin. This 63-amino acid protein stimulate binding of RNA I to RNA II and thus enhances the inhibitory action of RNA I. Inhibition of primer formation by RNA I in the presence or absence of the protein determines the copy number of a plasmid in a cell and the incompatibility between related plasmids.

Energy Conversion in Biology, Especially Muscle Contraction

A number of different topics have been studied under this project, the most important of which are the following. (a) An end of a microtubule (and probably an actin filament as well) undergoes a dynamic alternation between two distinct phases with radically different properties, depending on whether the end is capped with GTP or not. (b) Advances have been made on the theory of the control of metabolite concentrations, on the role of protons in chemiosmotic coupling theory, and on the incorporation of photochemical processes into the formalism of nonequilibrium thermodynamics.

Statistical Thermodynamics of Protein and Polynucleotide Systems

Two topics have been studied under this project: (a) Theoretical studies on the kinetics of relaxation of cruciformed DNA molecules in solution. (b) A one-dimensional piggy-back binding model for gyrase ATPase activity activated by long DNA molecules.

Regulation of Bacterial DNA Gyrase Synthesis by DNA Supercoiling

We have studied the regulation of DNA gyrase, the bacterial enzyme respo sible for DNA supercoiling. Synthesis of both the A and B subunits of DNA gyra in *E. coli* is itself controlled by DNA supercoiling, with much more enzyme being made from a relaxed DNA template than from a supercoiled one. We have also shown that topoisomerase I, whose DNA-relaxing activity balances the supercoiling produced by gyrase, is controlled in the opposite way; its synthesis is greatly reduced when DNA is relaxed. Each enzyme is thus preferentially synthesized when more of it is needed, enabling the cells to maintain control over the level of DNA supercoiling.

In order to study this mechanism of regulation in more detail, we have identified the promoter regions of the *gyrA* and *gyrB* genes, and have fused each promoter to the galactokinase gene in a plasmid vector. In these hybrid plasmids, which contain about 100 base pairs of the region surrounding the promoter, galactokinase synthesis is strongly stimulated by DNA relaxation. Thus the small region around the *gyrA* or *gyrB* promoter contains all the necessary information for this type of control. A deletion analysis has identified the necessary sequences even more closely, and is being pursued in order to locate the regulatory sites exactly.

Studies on the Mechanism of Genetic Recombination

The most recent developments in our study of the mechanism of genetic rearrangement reactions include the establishment of a cell free reaction system for the study of replicative transposition of bacteriophage Mu. The entire process of transposition reaction is faithfully reproduced in this reaction system: both cointegrates as well as simple inserts are produced, A and B gene products of phage Mu along with other bacterial proteins are required, and the substrate DNA has to carry two ends of Mu in their proper orientation. This is the first time the entire process of transposition reaction has been reproduced in a cell free reaction system.

The major findings obtained by using this reaction system include the following: there is strong evidence supporting the mechanism of transposition that involves a pair of strand transfer reactions at the initiation of the process The polarity of this strand transfer reaction has been determined. Mu A and B gene products have been purified to near homogeneity. Mu A protein was shown to bind specifically to the two end sequences of Mu DNA. Mu repressor, which is involved in regulation of life cycle of phage Mu, has been purified to near homogeneity. Mu repressor was shown to bind specifically to the operator region of Mu DNA. Involvement of Mu repressor in the direct regulation of transposition of phage Mu through functional interaction with Mu A protein is under investigation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 33000-18 LMB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study of Functions Involved in Genetic Recombination

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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	Anthony Maxwell	Visiting Fellow	LMB, NIADDK
	Rolf Menzel	Staff Fellow	LMB, NIADDK
	Kiyoshi Mizuuchi	Visiting Scientist	LMB, NIADDK
	Mary O'Dea	Chemist	LMB, NIADDK

COOPERATING UNITS *(if any)*

Professor David Friedman, University of Michigan, Ann Arbor, Michigan

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Section on Metabolic Enzymes

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	4	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have studied the regulation of DNA gyrase, the bacterial enzyme responsible for DNA supercoiling. Synthesis of both the A and B subunits of DNA gyrase in E. coli is itself controlled by DNA supercoiling, with much more enzyme being made from a relaxed DNA template than from a supercoiled one. We have also shown that topoisomerase I, whose DNA-relaxing activity balances the supercoiling produced by gyrase, is controlled in the opposite way; its synthesis is greatly reduced when DNA is relaxed. Each enzyme is thus preferentially synthesized when more of it is needed, enabling the cells to maintain control over the level of DNA supercoiling.

In order to study this mechanism of regulation in more detail, we have identified the promoter regions of the gyrA and gyrB genes, and have fused each promoter to the galactokinase gene in a plasmid vector. In these hybrid plasmids, which contain about 100 base pairs of the region surrounding the promoter, galactokinase synthesis is strongly stimulated by DNA relaxation. Thus the small region around the gyrA or gyrB promoter contains all the necessary information for this type of control. A deletion analysis has identified the necessary sequences even more closely, and is being pursued in order to locate the regulatory sites exactly.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 33006-06 LMB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on the Mechanism of Genetic Recombination

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kiyoshi Mizuuchi	Visiting Scientist	LMB, NIADDK
Others:	Toshiro Adachi	Guest Worker	LMB, NIADDK
	Robert Craigie	Visiting Fellow	LMB, NIADDK
	Michiyo Mizuuchi	Guest Worker	LMB, NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Section on Metabolic Enzymes

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The major object of this project is to uncover enzymatic steps involved in various genetic rearrangement reactions and to study the mechanism of action of the enzymes involved. The reactions studied include: the site specific recombination of bacteriophage lambda, resolution of the Holliday intermediate of general genetic recombination transposition-replication reaction of bacteriophage Mu, and rearrangement reaction of immunoglobulin genes. The most recent developments include the establishment of an in vitro reaction system for the study of replicative transposition of bacteriophage Mu. This in vitro reaction yields both cointegrate products as well as simple insertion products and requires both A and B gene products of phage Mu along with other bacterial proteins. The reaction requires a substrate DNA molecule that carries two ends of Mu in their proper relative orientation. These conditions are also the requirements for the Mu transposition-replication reaction in vivo. Thus, the in vitro reaction observed is the authentic Mu tarnsposition. This is the first time a transposition reaction has been demonstrated in a cell-free system.

By making use of this reaction system, we have obtained evidence that support the mechanism of transposition involving a pair of strand transfer reactions as the initiation step. The polarity of this strand transfer reaction has been determined. MuA and B gene products as well as the Mu repressor that is involved in the regulation of the process have been purified to near homogeneity. MuA protein was shown to bind specifically to the two end sequences of Mu DNA. Mu repressor was shown to bind specifically to the operator region of Mu DNA. Functional interaction between the two proteins were investigated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 33051-12 LMB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mammalian Enzymes Involved in Repair or Replication of DNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: S.B. Zimmerman Research Chemist LMB, NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Section on Physical Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Inactive.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 33054-09 LMB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structural Studies on Polynucleotides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S.B. Zimmerman	Research Chemist	LMB, NIADDK
Others:	B. Harrison	Chemist	LMB, NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Section on Physical Chemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Two publications based on earlier experimental work have been prepared and both have appeared:

1. A review of fiber diffraction studies in our laboratory which was presented at a Cold Spring Harbor symposium on DNA structure.

2. Results of a joint study with Dr. Sue Hanlon's group which indicated that DNA derivatized with n-butylamine yielded a B form in fibers at high relative humidity or in wetted fibers. This study used x-ray diffraction methods on DNA samples that had the distinctive circular dichroism spectrum repeatedly associated in the literature with the C form of DNA. We had questioned that association and, indeed, found that the DNA was in the B form--as expected from earlier studies in our laboratory.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 34001-19 LMB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Chromatin Structure and Function

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	G. Felsenfeld	Chief, Section on Physical Chemistry	LMB, NIADDK
Others:	W. Clough	Guest Worker	LMB, NIADDK
	B.M. Emerson	Staff Fellow	LMB, NIADDK
	M.M. Garner	Guest Worker	LMB, NIADDK
	H.J. Gould	Visiting Scientist and Expert	LMB, NIADDK
	D. Jackson	Chemist	LMB, NIADDK
	C. Lewis	Staff Fellow	LMB, NIADDK
	J.M. Nickol	Staff Fellow	LMB, NIADDK
	G. Sandeen	Chemist	LMB, NIADDK

COOPERATING UNITS (*if any*)

Donald C. Rau, Laboratory of Chemical Physics, NIADDK

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Section on Physical Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8	8	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

We have continued our studies of chromatin structure, in order to understand the forces that are involved in the packaging of DNA within eukaryotic nuclei, and to determine how that structure is modified in the neighborhood of genes that are being expressed. We have extended studies of the phasing of nucleosomes on DNA, and have found that for at least one arbitrarily chosen 145-base-pair DNA fragment, the placement of the histone octamer with respect to the ends of the DNA is asymmetric, showing that there are strong sequence-dependent forces of interaction between protein and DNA. In experiments concerned with the structure of chromatin containing transcriptionally active genes, we have examined the physical properties of nucleosome-like particles containing the chicken adult β globin gene, isolated from erythrocytes in which that gene is being expressed. We find that these particles possess properties very similar to those of normal nucleosome core particles, showing that the packaging of active genes at this level of organization is similar to that of bulk chromatin. We have also continued studies of the structure of DNA in the promoter region of this β globin gene, which we have found has a special chromatin structure when the gene is expressed, as demonstrated by an unusual sensitivity to nucleases. We have been able to reconstruct this sensitive structure by assembling chromatin on globin-gene containing plasmids in the presence of extracts from chicken erythrocyte nuclei. We have shown that these extracts contain one or more factors that bind specifically and tightly to the promoter region, and that appear to be responsible for generating the unusual chromatin structure of the region. The factor has been partially purified.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 34002-20 LMB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Enzyme Structure

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.R. Davies	Chief, Section on Molecular Structure	LMB, NIADDK
Others:	H. Savage	Visiting Associate	LMB, NIADDK
	R. S. Howland	Physicist	LMB, NIADDK
	J.L. Sussman	Special Expert	LMB, NIADDK
	G.H. Cohen	Research Chemist	LMB, NIADDK

COOPERATING UNITS *(if any)*

A. Wlodawer, National Bureau of Standards

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Section on Molecular Structure

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.75	2.75	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1. A molecular model of renin has been constructed based on its homology with the other aspartyl proteinases.

2. Crystals of tryptohpan synthetase have been examined and found to be very suitable for an X-ray diffraction investigation.

3. Neutron diffraction analyses have been carried out on bovine pancreatic trypsin inhibitor and on insulin. These studies reveal the exchangeable amide protons in the structure.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 34003-16 LMB

PERIOD COVERED

October 1, 1983 To September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Three-Dimensional Structure of Proteins of the Immune System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.R. Davies	Chief, Section on Molecular Structure	LMB, NIADDK
Others:	G.H. Cohen	Research Chemist	LMB, NIADDK
	E.W. Silverton	Research Chemist	LMB, NIADDK
	T.N. Bhat	Visiting Associate	LMB, NIADDK
	E.A. Padlan	Special Expert	LMB, NIADDK
	K. Saguna	Visiting Fellow	LMB, NIADDK

COOPERATING UNITS *(if any)*

Dr. S. Smith-Gill, NCI

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Section on Molecular Structure

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	4.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1. The crystals of the complex of an anti-lysozyme Fab and lysozyme have been grown to a size suitable for X-ray diffraction studies. X-ray data have been collected at the Cornell High Energy Synchrotron Source.

2. The phosphocholine-binding mouse myeloma Fab crystal structure has been refined completely. The final structure has been used to exaine the molecular nature of the phosphocholine binding site.

3. The structure of the galactan binding mouse myeloma protein J539 has been refined at 2.8 Å resolution. The structure is now being used to define the hapten combining site.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 35000-20 LMB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chemical and Structural Investigations of Nucleic Acids and Related Substances

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: H. Todd Miles, Chief, Section on Organic Chemistry LMB/NIADDK

OTHERS:	Frank B. Howard	Research Scientist	LMB/NIADDK
	Zu-kun Tan	Visiting Fellow	LMB/NIADDK

COOPERATING UNITS *(if any)*
Girjesh Govil, Tata Institute for Fundamental Research, Bombay, India

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Organic Chemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 3	PROFESSIONAL: 2	OTHER: 1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has the long-range objective of studying the chemistry and structure of nucleotides, polynucleotides and nucleic acids and the application of these studies to the understanding of biochemical and biological processes. Topics of current interest include: (1) structure and reactivity of polynucleotides and the relation between the two; (2) stabilization of ordered forms of nucleic acids and polynucleotides; (3) spectroscopic studies of polynucleotides: infrared, ultraviolet, and circular dichroism; (4) design and synthesis of new polynucleotides having properties required for the foregoing studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 35050-13 LMB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Replication, Recombination and Repair of Microbial DNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.-I. Tomizawa	Chief, Section on Molecular Genetics	LMB, NIADDK
Others:	T. Som	Visiting Associate	LMB, NIADDK
	S. Dasgupta	Visiting Associate	LMB, NIADDK
	H. Masukata	Visiting Associate	LMB, NIADDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Section on Molecular Genetics

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Replication of ColE1 DNA. Studies on DNA replication of plasmid ColE1 and its relatives have been continued. Transcripts by RNA polymerase (RNA II) that start the 555 nucleotides upstream of the replication origin of plasmid ColE1 extend beyond the origin. The hybridized transcript is cleaved by ribonuclease H at the origin and used as the primer for DNA synthesis by DNA polymerase I.

The primer formation is dependent on the secondary structure of RNA II. A single base change far from the replication origin can affect a specific stage of primer formation. Thus each single base-change affects initiation of synthesis of RNA II, hybrid formation between RNA II and the template DNA, the cleavage sites of RNA II by RNase H, the stability of association of RNA II or the use of cleaved RNA II as a primer by DNA polymerase I. Suppressor mutations that restore the activity of RNA II lost by a mutation have been found. Based on the results of analysis of mutant RNA IIs by partial digestion with an RNase we could deduce the structure of functional RNA II.

Primer formation is regulated by a plasmid-specified small RNA (RNA I). RNA I binds to RNA II at the complementary region. This binding results in inhibition of formation of the secondary structure necessary for primer formation. Based on the results of kinetic analysis of binding of RNA I to RNA II, we proposed a stepwise model of binding of RNA I to RNA II.

Primer formation is also regulated by a protein specified from a region downstream of the replication origin. This 63-amino acid protein stimulates binding of RNA I to RNA II and thus enhances the inhibitory action of RNA I. Inhibition of primer formation by RNA I in the presence or absence of the protein determines the copy number of a plasmid in a cell and the incompatibility between related plasmids.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 36002-10 LMB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Novel Recombination Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. L. Rosner	Research Biologist	LMB, NIADDK
Others:	R. Khanna	Visiting Fellow	LMB, NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Microbial Genetics

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Two loci, sapA and sapB, are involved in transpositional activation of the bgl operon in Escherichia coli. Deletion analyses and cloning experiments have located sapA next to the pgl gene and sapB near gal.

The survival of bacteria carrying the drug-resistance transposons Tn5 or Tn9 on media with high concentration of kanamycin or chloramphenicol, respectively, is enhanced over 20-fold by a variety of bacterial repellants, including acetate, benzoate, salicylate and dimethyl-sulfoxide.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 36051-16 LMB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetics and Structure of the Oncogenic Virus, SV40

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert G. Martin	Chief, Section on Microbial Genetics	LMB, NIADDK
Others:	J. M. Nickol	Senior Staff Fellow	LMB, NIADDK
	R. L. Lechner	Senior Staff Fellow	LMB, NIADDK
	C. Nobile	Visiting Fellow	LMB, NIADDK
	B. S. Rao	Visiting Fellow	LMB, NIADDK
	S. S. Wang	Chemist	LMB, NIADDK
	Y. M. Johnson	Biological Laboratory Technician	LMB, NIADDK

COOPERATING UNITS *(if any)*

David Livingston, Sidney Farber Cancer Institute, Massachusetts
Daniel Tenan, Sidney Farber Cancer Institute, Massachusetts

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Microbial Genetics

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	4.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have isolated a number of clones believed to contain the origins of DNA replication in monkey cells. A detailed analysis of nine of these has been completed. Only two contain unique sequences. The remaining seven contain moderately reiterated or highly reiterated sequences. One moderately reiterated monkey sequence of 196 bp (base pair) has been found in two different clones. All of the sequences contain extensive AT-rich regions and potential stem-loop structures. Six of the nine share between 13 and 19 bp of a 21 bp "consensus sequence." Four of five tested replicate preferentially early in S phase. None has been shown to act as a plasmid replication origin.

In other experiments, we have generated deletions and substitutions of the 27 bp perfect inverted repeat sequence (13x1x13) at the SV40 replication origin.

A substitution mutant, in which an entirely different 13x13 perfect repeat has been substituted for the original 13x1x13 sequence, is incapable of binding the SV40 T antigen. Heteroduplex molecules between the wild type and mutant molecule obligatorily exist as cruciform structures, and on reconstitution with histones cause nucleosome phasing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 36101-10 LMB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Energy Conversion in Biology, Especially Muscle Contraction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*
PI: T. L. Hill Chief, Section Theoretical Molecular Biology LMB/NIADDK

COOPERATING UNITS *(if any)*
Marc W. Kirschner, University of California, San Francisco
Giuseppe Inesi, University of Maryland, Baltimore
Marie-France Carlier, CNRS, Gif-sur-Yvette, France

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Theoretical Molecular Biology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	2.2	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A number of different topics have been studied under this project, the most important of which are the following. (a) An end of a microtubule (and probably an actin filament as well) undergoes a dynamic alternation between two distinct phases with radically different properties, depending on whether the end is capped with GTP or not. (b) Advances have been made on the theory of the control of metabolite concentrations, on the role of protons in chemiosmotic coupling theory, and on the incorporation of photochemical processes into the formalism of non-equilibrium thermodynamics.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 36102-13 LMB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders)*
Statistical Thermodynamics of Protein and Polynucleotide Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. L. Hill	Section Chief	LMB, NIADDK
Others:	Y. Chen	Research Chemist	LMB, NIADDK
	H. V. Westerhoff	Visiting Associate	LMB, NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Theoretical Molecular Biology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0.8	0.8	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Two topics have been studied under this project: (a) Theoretical studies on the kinetics of relaxation of cruciformed DNA molecules in solution. (b) A one-dimensional piggy-back binding model for gyrase ATPase activity activated by long DNA molecules.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 36104-03 LMB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Thermal Measurements of Biomolecular Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.D. Ross	Research Chemist	LMB, NIADDK
Others:	A.C. Steven	Visiting Scientist	LPB, NIADDK
	F.B. Howard	Chemist	LMB, NIADDK
	T. O'Leary	Medical Staff Fellow	LCP, NIADDK
	I. Levin	Chemist	LCP, NIADDK

COOPERATING UNITS *(if any)*

Andrew Shrake, BB, DBBP
Lindsay W. Black, University of Maryland Medical School
Baltimore, Maryland

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Section on Physical Chemistry

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Thermodynamic characterization, obtained from calorimetric techniques, has been carried out in the following areas:

Proteins: Stability of defatted human serum albumin and the effect of long-chain fatty acids.

Nucleic Acids: Energetics of the B → Z transition of DNA and the contribution of the 2-amino group of adenosine to the stability of two-stranded RNA helicies has been determined.

Phospholipid Membranes: The effect of anesthetics on the various phase transitions of dipalmitoylphosphatidylcholine bilayers has been delineated.

Macromolecular Assembly Processes: The thermodynamic parameters of the expansion of the surface lattice in microtubules of the T4 bacteriophage capsid protein, gp23*, have been determined and interpreted at the molecular level.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 36105-02 LMB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Influences of Macromolecular Crowding on Biochemical Systems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S.B. Zimmerman	Research Chemist	LMB, NIADDK
Others:	B. Harrison	Chemist	LMB, NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Section on Physical Chemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	0.9	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To our initial studies of the effects of macromolecular crowding on enzymatic DNA joining, we noted that T4 DNA ligase could be enormously stimulated by high concentrations of polymers. We here extend those studies to a variety of DNA substrates.

The rates of blunt-end and cohesive-end ligation of DNA by T4 DNA ligase are increased by orders of magnitude in the presence of high concentrations of a variety of nonspecific polymers such as polyethylene glycol, Ficoll, bovine plasma albumin, or glycogen. Blunt-end ligation of small self-complementary oligodeoxyribonucleotides is also stimulated. The use of polyethylene glycol 6000 in such systems is characterized in some detail. Conditions are suggested which either greatly increase the rate of formation of and size of linear ligation products or which allow smaller but significant improvements in the amounts of circular ligation products.

A patent covering these results on polymer-stimulated enzymatic ligation of DNA was applied for on behalf of the government.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 36106-02 LMB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of Digestive Enzymes in the Intestine of C. elegans

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: J.D. McGhee Visiting Scientist, LMB, NIADDK

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Section on Physical Chemistry

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Terminated.

Annual Report of the Arthritis and Rheumatism Branch
National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases
October 1, 1983 through September 30, 1984

The Branch's professional activities include both clinical and laboratory investigations. The clinical involvement is substantial: During the past year there were some 300 admissions (average length of stay 14 days) accounting for over 5,000 patient-days. Over 2,000 follow-up outpatient visits were scheduled including approximately 100 evaluations of new patients. In addition, ARB physicians responded to some 100 requests for consultation at the Clinical Center. The clinical investigations involve the collection and study of tissue and blood specimens as well as trials of various therapeutic regimens, chiefly on patients with systemic lupus erythematosus and rheumatoid arthritis. In order to assure the smooth functioning of this heavy clinical program, Dr. John H. Klippel was designated as Clinical Coordinator for the Branch.

The laboratory investigations involve studies on animal models of lupus and arthritis as well as fundamental studies at the cellular and molecular level, on various aspects of the immune response and inflammatory processes.

Dr. Henry Metzger was invited to present the Ecker Lecture at Case Western University and received the Joseph Mather Smith Prize from Columbia. Drs. Thomas J. Santoro (preceptor A.D. Steinberg) and Dr. Daniel G. Malone (preceptor R. Wilder) were awarded Senior Rheumatology Scholar Awards by the American Rheumatism Association.

I. Disease Related Studies

A. Rheumatoid Arthritis

1. Pathogenesis

a. Animal Models

When systemically injected into rats, guinea pigs, and rabbits, bacterial cell walls can induce a chronic erosive synovitis, the clinical and histological features of which resemble human rheumatoid arthritis. Current studies (48)* are directed towards defining factors that influence the incidence, type and severity of the arthritis, the mechanisms that lead to tissue injury, and modulating procedures.

Among the determining factors is the bacterial material itself. The peptidoglycan from Streptococcal Groups A, B and C as well as selected lactobacilli which are arthritogenic all contain large amounts of rhamnose in their group specific carbohydrate and are resistant to degradation by lysozyme. The size of the fragments injected influences the latency period and duration of the disease. The injected material localizes to the synovia of peripheral joints, spleen, liver and bone marrow.

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*Numbers in parentheses refer to last digits of project number, e.g. Z01 AM 41

Within 24 hr an acute inflammation of the joints occurs. In susceptible strains a chronic phase begins as early as 8 days or as late as several weeks after the acute inflammation and is characterized by proliferation of cells in the synovial lining, with villus formation. Tissue from such lesions produces high levels of several mediators (interleukin 1, prostaglandin E, fibroblast growth factor) derived from mononuclear cells. Hyperplasia of the central and peripheral lymphoid organs is also observed. The localization of the bacterial cell walls and the acute phase is similar in all animals but the incidence and severity of the chronic phase is markedly influenced by the sex of the animals, and several other genes including those of the major histocompatability locus. Interestingly, when the thymus is removed from rats of susceptible strains, the acute arthritis occurs but the process resolves over 2 weeks without development of a chronic phase.

b. Human Disease

In order to understand better the pathological changes in human rheumatoid arthritis, biopsies of synovial tissue obtained by direct visualization from affected joints are being studied (66). It has been found that such specimens can be classified into three meaningful groups:

Specimens in Group A show diffuse lymphocytic infiltration predominantly of T-cells bearing the so-called OKT4/Leu 3 surface antigens - markers associated with inducer/helper cells. HLA-Dr + cells associated with the OKT8 antigen - the latter a marker for suppressor cells - are also seen. Such synovia spontaneously secrete large amounts of monocyte chemotactic factors. Patients having such synovial infiltration often show cutaneous anergy, i.e. they are hyporesponsive to locally injected soluble antigens. These patients have an increased number of T lymphocytes in their blood bearing HLA-Dr antigens - i.e. presumably activated T cells.

Synovia of "Group B" show markedly less lymphocytic infiltrates and those cells present are prinicpally of the OKT8+ group. The specimens produce much less chemotactic factor than those of Group A. The patients in this group do not express cutaneous anergy and their peripheral blood shows fewer HLA-Dr + cells.

Synovia of "Group I" show an intermediate pattern compared to Groups A and B and this is also true of the other features cited above.

The synovial patterns show a striking correlation with clinical responsiveness to apheresis (Sec. IA 2b iv)

2. Therapeutic trials

The Branch is involved in testing several therapeutic regimens for rheumatoid arthritis:

a) Low, "pulse" doses of oral methotrexate are being administered to 25 outpatients with poorly controlled arthritis in a double-blind protocol as part of a cooperative study with ten other clinics under the direction of the Cooperating Clinics for the Systemic Studies of Rheumatic Diseases (CCSRD) (57). Preliminary analyses indicate that methotrexate is more efficacious than placebos but also leads to significant hepatic and other toxicity.

b) Treatment with high "pulse" doses of intravenous methylprednisolone is being tested in an open pilot study to determine if the benefit/risk ratio can be improved (53). Preliminary assessment suggests that this form of therapy warrants further study.

c) Treatment with azathiaprine or d-penicillamine is being studied in patients unresponsive or intolerant to gold therapy under a double-blind protocol in collaboration with ten other clinics under the direction of CCSRD (49). Data analysis on some 200 patients enrolled in the study is underway.

d) Leukapheresis of 14 patients unresponsive to conventional therapy has been tested (54). No correlation between responsiveness and initial disease activity was observed. However, a striking correlation was observed between responsiveness and the synovial histology: six of seven "Group A" patients (see A1 b above) showed a greater than 40% decrease in "articular index" and these showed a marked decrease in anergy. The duration of improvement was variable: 2 weeks to more than 6 months. No Group B patients showed improvement by either clinical or laboratory criteria.

The laboratory investigations taken together with the clinical trial indicates that clinically similar patients can be meaningfully segregated. This has implications for the etiology and treatment of their disease and deserves further analysis.

B. Systemic Lupus Erythematosus (SLE)

1. Pathogenesis

a. Animal models (20)

Inbred mice manifest a spectrum of syndromes that closely resemble the variety of disease manifestations observed in human SLE. Study of such mice has shown that there is no single autoimmune gene. Rather, full expression requires at least six discrete loci. Even individual manifestations can be shown to require more than a single gene. For example, one of the underlying characteristics in autoimmune mice is a heightened B cell activity. CBA/N mice bear a sex-linked gene - called xid - which interferes with B cell activation. It is of interest that several different autoimmune congenic mice bearing the xid gene fail to develop the serologic and clinical manifestations of autoimmunity.

Recently, increased expression of certein "oncogenes" - genes activated in neoplastic conditions - has been observed in some of the autoimmune mice. This association of specific genes with the autoimmune state deserves further study.

Environmental factors also play an important role. For example, mice not destined to develop spontaneous morbidity can be induced to develop rapidly progressive autoimmune disease when administered polyclonal B cell activators. Contrariwise, autoimmune strains fail to develop tolerance to orally administered antigens (20). This defective gastrointestinal tolerance could conceivably contribute to the development or perpetuation of autoimmunity due to the elicitation of antibodies to exogenous antigens that might cross-react with self components. Although immune complexes are strongly implicated as an important factor in the pathology of autoimmune disease the mechanism is unclear. Using model immune complexes one can show abnormalities in the handling of such complexes in autoimmune mice - even prior to other manifestations or disease (18). Nevertheless, treatment of such animals with drugs known to ameliorate the disease appears not to affect the the handling of such model complexes.

b. Human disease

i. Renal involvement

One of the principal serious complications of SLE is renal involvement. A detailed analysis of renal pathology as manifested in biopsy specimens, has been initiated (38). The principal findings are that active disease may be evident on biopsy even when blood chemistries are normal. Biopsies showing diffuse proliferative glomerulonephritis show a broad spectrum of morbidity. The concomitant presence of sclerosing lesions appears to be an important prognostic feature.

Ongoing studies (65) in which patients are placed into one of five categories on the basis of renal involvement, are continuing. The major objective is to collect data systematically in order to understand the course of illness prior to the development of major renal disease so that therapeutic protocols directed towards preventing such involvement can be devised. Data collection continues.

ii. Disorders of immune regulation

Patients with SLE have enhanced spontaneous B cell activity but depressed responses to certain mitogens when their cells are tested *in vitro*. Nevertheless, *in vitro* responses to certain T cell-dependent antigens were found to be normal. Recent results showing that such antigens fail to stimulate normal amounts of suppressor cells *in vitro* might explain the enhanced acitivty of B cells in the disease (52).

Studies with monoclonal antibodies to distinctive cell surface markers indicate that some patients have a decrease in suppressor cells and their precursors (23). Others have a high suppressor:helper cell ratio although this appears to result chiefly from a defect in the helper cells. Patients showing the latter picture have a high incidence of renal disease, thrombocytopenia and lymphopenia whereas those having fewer than normal suppressor cells tend to have multisystem disease with muscle involvement, lymphadenopathy. sicca syndrome and later onset of their disease.

Several cytotoxic responses are severely depressed in patients with SLE - a defect correctable *in vitro* by addition of interleukin 2. These phenomenon are being explored in depth with cells from patients.

Lymphocytes of patients with SLE but not normals release low molecular weight DNA which is enriched in guanine and cytosine. These materials are highly immunogenic when tested in mice and a hybridoma antibody prepared by fusing of B cells from a patient showed reactivity with such material (21). The latter may be an important source of immunogenic DNA and DNA in immune complexes. Such complexes can cause tissue damage where they localize. There is evidence that the nature of the antigen is an important factor in determining the site of such localization (62).

A defect in the ability of the reticulo-endothelial system to deal with particulate antigens was shown in the past but recent data demonstrate that this is not simply due to overload of receptors for the Fc region of IgG. Indeed, the peripheral blood monocytes from such patients show an increased capacity to bind immunoglobulins (60).

2. Therapeutic studies

a) Animal models

That different inbred strains of mice show different patterns of morbidity due to their autoimmune disease, provides an opportunity to test whether different therapeutic regimens might be more beneficial in some and not others. This appears to be the case with treatment with cyclophosphamide (22). On the basis of such studies it may be possible to identify patients that are more likely to respond to a given therapy.

Recent studies have indicated that a diet enriched in polyunsaturated eicosapentaenoic acid prolonged the life of (NZB x NZW) F1 autoimmune mice, when given before or after the onset of their disease (22). On the basis of these findings a protocol has been established for assessing the use of such diet supplements in patients.

b) Human disease

Current therapeutic regimens being investigated include the use of intermittent intravenous methylprednisolone or cyclophosphamide in addition to low doses of oral corticosteroids, in patients with renal involvement (56). Approximately 40 patients have been enrolled in this study so far. The study is designed to allow for long-term follow-up.

C. Other Connective Tissue/AutoImmune Diseases

1. Pathogenesis

In various autoimmune diseases patients (and animals) develop characteristic patterns of autoantibodies. There is evidence that some of these may arise because of specific interactions between an exogenous agent (virus, bacterium, drug) and the sites to which the antibodies bind.

A search has been begun (69) to determine whether certain picornaviridiae can be implicated in the causation of inflammatory myositis and the antibodies to amino acid t-RNA synthetases which are characteristic in such cases. Mice are being infected with picornaviruses and their sera and tissues tested. In addition, sera of patients with myositis and other autoimmune diseases are being screened for antibodies to the t-RNA synthetases.

2. Therapeutic trials

Goodpasture's Syndrome is usually characterized by rapidly progressive glomerulonephritis due to autoantibodies directed towards the glomerular basement membrane. A multi-center prospective control study has been initiated (59) in which patients with such antibodies will be treated with various drug regimens with or without concomitant plasmapheresis.

II. Fundamental Studies

A. Studies on Fc Receptors

Antibodies and antibody-like molecules are the only molecules known to mediate the fine specificity associated with the immune response. These molecules act as receptors on cell surfaces or in solution, where they activate so-called biologic effector systems. An understanding of the precise mechanism of action may allow one to develop therapeutic manipulations of the immune system.

One such system that is being investigated in depth is the IgE - mast cell system (25). Current studies are directed towards defining the receptor (Fc receptor) on mast cells with which the IgE interacts and stimulation of which leads to release of mediators importantly involved in allergic and inflammatory reactions. Recently, progress has been made in the isolation of structurally intact receptors and defining the principal factors that stabilize the structure. Studies on the earliest stages of activation have implicated a channel specific for calcium and investigations concerning the relationship of these channels to the receptors have been initiated.

Concomitant with stimulation of secretion, the activation of the receptors leads to their own redistribution. Recent work (51) suggests that the internalization is a discrete event and is unlikely to be directly responsible for or a consequence of the stimulation of exocytosis. The cell appears to be able to distinguish between receptors with specificity for different immunoglobulins and the mechanisms for this discrimination are being sought.

B. Mononuclear Cells

Preliminary studies on peripheral blood mononuclear cells from patients with rheumatoid arthritis (67) show that by various functional criteria such cells are different than cells from healthy individuals. Studies have been initiated in which large numbers of mononuclear cells are obtained from normal subjects by leukapheresis characterized by a variety of structural and functional criteria: Fc receptors, lymphokine production, challenge with antigens or allogeneic cells, etc.

C. Complement

The classical and alternative complement pathways participate in the effector functions induced by the interaction of antibodies with antigen. Components of these may also influence the regulation of immune responses. Thus, it has recently been found that a product of C'3 - C'3b - can inhibit responses of B cells by direct action on the cells. Factor H (BIH), a regulatory protein of the alternative pathway has similarly been found to inhibit B cell differentiation but not proliferation. Complement also appears to affect T cells since the breakdown products C3b and C3d can enhance human T-cell cytotoxic responses. Contrariwise, antibodies to the receptors for certain complement components appear to block cytotoxic reactions examined *in vitro* (68).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 41018 10 ARB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Production of covalently cross-linked immune complexes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. Paul H. Plotz Senior Investigator ARB/NIADDK

Leslie Schrieber Visiting Associate ARB/NIADDK

William Mullins Med. Staff Fellow ARB/NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Disease

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS: 2	PROFESSIONAL: 2	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies underway continue earlier observations on immune complex biology using site-specifically cross-linked model complexes. Two major aspects are under study at the moment. First, the hepatic handling of model complexes in auto-immune mice is being studied in detail using organ perfusion. This approach allows a more detailed and subtle understanding than whole animal studies. Auto-immune mice show an abnormality in handling complexes before they are clinically ill. Second, in studies underway, the influence of drugs which are known to ameliorate auto-immune disease upon immune complex handling in normal and auto-immune mice, is being explored.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 41020-17 ARB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathogenesis of Autoimmunity in Mice with SLE-like Illness

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Alfred D. Steinberg, Chief, Cellular Immunology Section, NIADDK
Yvonne J. Rosenberg, Visiting Associate, Cellular Immunology Section, NIADDK
Thomas J. Santoro, Medical Staff Fellow, Cellular Immunology Section, NIADDK
Dennis M. Klinman, Medical Staff Fellow, Cellular Immunology Section, NIADDK
John D. Mountz, Medical Staff Fellow, Cellular Immunology Section, NIADDK
Howard R. Smith, Medical Staff Fellow, Cellular Immunology Section, NIADDK
Michael L. Miller, Guest Researcher, Cellular Immunology Section, NIADDK

COOPERATING UNITS *(if any)*

Lyn Yaffe, Pathologist, NMRI, Bethesda, MD
J. F. Mushinski, Senior Investigator, NCI
Thomas M. Chused, Senior Investigator, LMI, NIAID

LAB/BRANCH

Arthritis and Rheumatism

SECTION

Cellular Immunology

INSTITUTE AND LOCATION

NIADDK - Bldg. 10, Rm. 9N-218

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	2	3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Autoimmunity in New Zealand mice has a genetic and immune basis; however, expression of severity can be modified by viral and other environmental factors as well as sex hormones. The immune factors are complex. These include excessive B-cell activation and abnormal T cell function.

A single gene, xid, prevents disease in congenic NZB and (NZB x NZW) F_1 and BXSB mice, and markedly retards disease in MRL-lpr/lpr mice. BXSB mice have a Y chromosome-linked gene which accelerates autoimmunity in autoimmune mice, but does not cause it in non-autoimmune mice. This gene requires an autosomal gene from BXSB mice for full expression in NZB mice, but not in NZW mice.

All of the autoimune strains studied have a defect in experimental tolerance. In NZB mice, the defect maps to a pre-T stem cell. Small numbers of NZB cells interfere with the development of tolerance suggesting that they secrete a factor which causes lymphocyte activation rather than supression.

Analysis of cytokines produced by autoimmune mice has led to a first level of appreciation of the importance of low molecular weight mediators of cellular communication in the immune abnormalities of autoimmune mice. The defects in autoimmune mice can be mimicked in normal mice by appropriate manipulation (removal of regulatory T cell function and chronic immune stimulation).

Studies of gastrointestinal immunity reveal that ingestion of certain food may fail to induce tolerance normally or even induce an immune response. Therefore, food in the diet of certain patients could trigger immune responses.

Different autoimmune mice have increased expression of different oncogenes. lpr/lpr and gld/gld mice have increased myb and raf RNA. The xid gene prevents increased ras expression, suggesting that that oncogene is associated with the Lyb 5^+ subset and autoantibody production.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 41021-16 ARB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Antinuclear Antibodies in Spontaneous and Drug-induced SLE and Other Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Alfred D. Steinberg, Chief, Cellular Immunology Section, NIADDK
Howard R. Smith, Medical Staff Fellow, Cellular Immunology Section, NIADDK
Michael L. Miller, Guest Researcher, Cellular Immunology Section, NIADDK

COOPERATING UNITS *(if any)*

Elizabeth S. Raveche, Senior Investigator, LEP, NIADDK
H. Sano, Harvard Medical School
C. Morimoto, Harvard Medical School

LAB/BRANCH

Arthritis and Rheumatism

SECTION

Cellular Immunology

INSTITUTE AND LOCATION

NIADDK - Bldg. 10, Rm. 9N-218

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5	3	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Antibodies to nucleic acids are important diagnostically and pathogenetically in systemic lupus. Patients with active SLE have an increase in DNA in immune complexes. Such DNA is enriched in guanine (G) and cytosine (C). Low molecular weight DNA enriched in G-C is releaed from SLE leukocytes. This DNA may be important in the pathogenesis of SLE. Genes favoring the production of anti-DNA may include those which lead to the release of immunogenic DNA.

In mice a subset of B cells is responsible for anti-DNA production. A similar human subset is being sought. If found, an attempt could be made to preferentially eliminate such B cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 41022-13 ARB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Therapeutic Studies in Murine Lupus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Alfred D. Steinberg, Chief, Cellular Immunology Section, NIADDK
Howard R. Smith, Medical Staff Fellow, Cellular Immunology Section, NIADDK

COOPERATING UNITS *(if any)*

D. Robinson, Harvard Medical School
J. Prickett, Harvard Medical School

LAB/BRANCH

Arthritis and Rheumatism

SECTION

Cellular Immunology

INSTITUTE AND LOCATION

NIADDK - Bldg. 10, Rm. 9N-218

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A diet enriched in a polyunsaturated fatty acid, eicosapentaenoic acid, markedly retards the development of autoimmunity in (NZB x NZW) F_1 females. This works even after the onset of disease. Human trials are being designed.

Cyclophosphamide is effective treatment in a variety of autoimmune strains unrelated to the cellular basis of illness or the nature (B or T cell) of the proliferating cells. This therapy is effective against the lymphadenopathy of MRL-lpr/lpr mice and the coronary artery disease of BXSB and (NZW x BXSB)F_1 mice as well as the renal disease of most examples of murine lupus. However, when all of the strains are thrown into a single study (analogous to human studies) efficacy is harder to demonstrate. Cyclophosphamide markedly reduces myb RNA in MRL-lpr/lpr mice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 41023-10 ARB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study of Immune Abnormalities in Patients with Systemic Lupus Erythematosus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Alfred D. Steinberg, Chief, Cellular Immunology Section, NIADDK
Thomas J. Santoro, Medical Staff Fellow, Cellular Immunology Section, NIADDK
Masaaki Honda, Visiting Fellow, Cellular Immunology Section, NIADDK
Howard R. Smith, Medical Staff Fellow, Cellular Immunology Section, NIADDK

COOPERATING UNITS *(if any)*

C. Morimoto, Harvard Medical School
Yves Borel, Harvard Medical School
Thomas M. Chused, Senior Investigator, LMI, NIAID

LAB/BRANCH

Arthritis and Rheumatism

SECTION

Cellular Immunology

INSTITUTE AND LOCATION

NIADDK - Bldg. 10, Rm. 9N-218

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients with SLE have excessive B cell proliferation. They have increased proliferation of bone marrow stem cells and circulating B cell precursors. Disease activity is characterized by a switch from just proliferation to differentiation and immunoglobulin synthesis. In some patients the T cell regulation of B cell functions is abnormal. In an attempt to better understand cell-cell interactions, normals were studied for the capacity of T cells to respond to autologous non-T cells. IL 2 production by $T4^+$ cells was necessary for the proliferation of $T8^+$ (suppressor) cells. This function is subnormal in patients with SLE.

Monoclonal antibodies were used to study subpopulations of SLE patients. One subset, with a reduced ratio of helper/suppressor cells, was characterized clinically by renal disease, thrombocytopenia, leukopenia and early age of onset of disease. Another subset had a high ratio and a clinical picture which included muscle and lung disease, Sicca syndrome, lympadenopathy and CNS disease. Thus, SLE may not be a single disease, but a group of syndromes with different genetic and cellular bases.

SLE patients have a variety of T cell defects. These are largely explained by anti-T cell antibodies produced by the B cells of these patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41025-13 ARB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on the cell surface receptor for IgE

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. Metzger,	Chief, Sec. Chem. Immunol.	ARB/NIADDK
	G. Alcaraz	Guest Researcher	ARB/NIADDK
	R. Quarto	Visiting Fellow	ARB/NIADDK
	J.P. Kinet	Visiting Associate	ARB/NIADDK
	Y. Takagaki	Visiting Associate	ARB/NIADDK

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Chemical Immunology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 6	PROFESSIONAL: 6	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are pursuing an in-depth investigation on the structure and function of the membrane receptor for immunoglobulin E (IgE). The principal cells with which we are working is a line of rat basophilic leukemia cells which can be grown in vivo as solid tumors or in vitro. During the past year we have pursued the following studies: 1) an evaluation of those conditions that promote or inhibit the dissociation of the subunits of the receptors 2) further characterization of the subunits 3) development of probes to identify the genes of the receptors 4) induction of monoclonal antibodies to the subunits of the receptors 5) studies on the putative ion channels induced as an early stage of activation of the receptors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41038-07 ARB

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Renal Biopsy Pathology in Systemic Lupus Erythematosus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

J. E. Balow, Senior Investigator, ARB, NIADDK
H. H. Austin, Expert, CC

COOPERATING UNITS *(if any)*

Armed Forces Institute of Pathology, Washington, DC

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: .25	PROFESSIONAL: .25	OTHER:

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Detailed analysis of renal biopsy pathology has been undertaken in patients with systemic lupus erythematosus who are receiving various therapeutic regimens of immunosuppressive agents. Biopsies will be scored for various indices of pathological activity as well as for patterns of deposition of immune complexes by immunofluorescence and electron microscopy; these morphologic parameters are to be related to renal function data in order to ascertain whether more accurate prognostic indices can be developed and whether more sensitive measures of therapeutic response can be identified.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 41040-12 ARB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study of various cytotoxic drug programs in diffuse lupus nephritis (PACIV)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Alfred D. Steinberg, Chief, Cellular Immunology Section, NIADDK
Paul H. Plotz, Senior Investigator, ARB, NIADDK
John H. Klippel, Senior Investigator, ARB, NIADDK
James E. Balow, Senior Investigator, ARB, NIADDK
Ronald L. Wilder, Senior Investigator, ARB, NIADDK

COOPERATING UNITS *(if any)*

John L. Decker, Director, Clinical Center
Howard A. Austin, Attending Nephrologist, Clinical Center

LAB/BRANCH

Arthritis and Rheumatism

SECTION

Cellular Immunology

INSTITUTE AND LOCATION

NIADDK - Bldg. 10, Rm. 9N-218

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	1	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients with the diffuse forms of systemic lupus erythematosus nephritis previously entered into therapeutic trials continue to be evaluated. Long-term studies suggest that the degree of scarring on the renal biopsy may provide important prognostic information. The subset of patients with a mild degree of scarring responds to oral azathioprine or oral cyclophosphamide when added to low doses of corticosteroids. Patients with a greater degree of scarring are not effectively treated with those drug programs, but may respond to intravenous boluses of cyclophosphamide. The relative effectiveness and toxicity of various drug programs continues to be systematically evaluated. Patients aged 8-23 years are at greater risk of developing renal functional deterioration than are older patients with SLE and nephritis. Long-term follow-up of patients with lupus nephritis is critical to proper therapeutic evaluation. Such study has begun to demonstrate the efficacy of bolus cyclophosphamide (0.5 - 1.0 G/M2/month) over daily oral cyclophosphamide with much decreased toxicity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES • PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41048-05 ARB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of Genetic and Pathophysiologic Features in the Rat of Experimental Synovitis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: R.L. Wilder, Senior Investigator, ARB/NIADDK
P.H. Plotz, Senior Investigator, ARB/NIADDK
J.B. Allen, Biochemist, ARB/NIADDK
T.J. Lehman, Medical Staff Fellow, ARB/NIADDK

COOPERATING UNITS *(if any)*

Laboratory of Microbial Immunology, NIDR

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Diseases

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 2	PROFESSIONAL: 1.5	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Chronic Erosive Synovitis can be induced experimentally in rats by systemic administration of aqueous suspensions of cell walls from certain bacteria, such as Group A streptococci and lactobacilli. Host genetic and sex-linked hormonal factors, as well as cell wall factors, influence incidence and severity of acute and chronic disease. Arthritogenic cell walls are lysozyme resistant and are rich in rhamnose. The development of arthritis in susceptible rat strains is dependent, in variable degrees, upon deposition and persistence of the cell walls in synovial tissues. Acute arthritis reflects a direct pro-inflammatory toxic effect of the cell walls at their sites of deposition. The development of chronic arthritis is dependent upon thymus-derived lymphocytes. The chronic arthritis can be suppressed by treatment with the synthetic retinoid, 4-hydroxyphenyl-retinamide, and the immunoregulatory agent, cyclosporin A. This animal model provides a powerful tool to investigate mechanisms that regulate susceptibility and resistance to chronic inflammation, as well as, mechanisms that produce tissue injury. Potential new therapeutic agents may also be studied.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01- AM 41049-05 ARB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Comparative efficacy and safety of azathioprine and d-penicillamine for RA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. Paul H. Plotz	Senior Investigator	ARB/NIADDK
J.H. Klippel	Senior Investigator	ARB/NIADDK
R. Wilder	Senior Investigator	ARB/NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Disease

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In this study patients with rheumatoid arthritis unresponsive or intolerant to gold therapy will be randomized in a double-blind trial to receive either azathioprine or d-penicillamine. The trial will compare the efficacy and toxicity of these during a 24 week trial and an extended follow-up observation period. The Arthritis and Rheumatism Branch is one of eleven clinics carrying out this protocol under the direction of the Center for Cooperative Studies in the Rheumatic Diseases.

THIS PROJECT HAS BEEN TERMINATED

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
ZO1 AM 41050-04 ARB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)* Comparative efficacy and safety of myochrysine and auranofin in the treatment of rheumatoid arthritis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. Paul H. Plotz	Senior Investigator	ARB/NIADDK
J.H. Klippel	Senior Investigator	ARB/NIADDK
R. Wilder	Senior Investigator	ARB/NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Disease

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Project terminated

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM41051-05 ARB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Natural History and In Situ Organization of the Receptors for IgE

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Pnncipal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. C. Isersky-Carter	Senior Investigator	ARB/NIADDK
J. Rivera	Biologist	ARB/NIADDK
K. Furuichi	Visiting Fellow	ARB/NIADDK

COOPERATING UNITS *(if any)*
LMI, NIDR,
LP, NCI

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Chemical Immunology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 4	PROFESSIONAL: 3	OTHER: 1

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are investigating the natural history of the receptors for immunoglobulin E (IgE) and immunoglobulin G (IgG) on mast cells and basophils. We aim to study the mode of function and turnover of the free and ligand bound receptors and the fate of the cell bound IgE before, during and after exocytosis of mediators of the allergic response which is initiated by aggregation of surface bound IgE. We found that monomeric IgE molecules remain resident on the cell surface for over 250 hours and endocytosis is only affected by cross-linking of surface bound molecules. The minimal size aggregate required to signal internalization was dimeric. Interestingly, dimers provide only a poor signal for histamine release from RBL cells. Internalized IgE is later released, the receptor internalizes with the ligand and does not appear to be reusable. Unlike the process of mediator release, internalization of aggregated IgE by RBL and normal mast cells is independent of calcium and proceeds at a different rate. The rate of internalization is the same for normal mast cells and RBL cells. Empty receptors for IgE and monomeric IgE cointernalize with crosslinked IgE. Endocytosis and coendocytosis could account, in part, for the phenomenon of desensitization. Cell bound aggregated IgG is endocytosed together with some empty receptors. When cells are allowed to bind aggregated IgE as well as IgG, the endocytosis of either is unaltered. Additionally, endocytosis of aggregated IgG has no effect on monomerically bound IgE. Unlike, the receptor for IgE, the receptor for IgG might be multimeric. It is hoped that further clarification of these aspects of mast cell and basophil function will lead to better understanding of the allergic response.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 41052-05 ARB

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Disorders of Immune Regulation In Patients with Systemic Lupus Erythematosus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

J. E. Balow, Senior Investigator, ARB, NIADDK
G. C. Tsokos, Visiting Scientist, ARB, NIADDK
G. Inghirami, Visiting Fellow, ARB, NIADDK
P. C. Smith, Biologist, CC

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	.75	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients with systemic lupus erythematosus have been found to have various disturbances of the cell-mediated immune response. Cellular aberrations include enhanced spontaneous B cell activity with abnormal triggering in vitro, deficient immunoregulatory T cell circuits, deficient cytotoxic responses including natural killer cell activity, alloantigen and viral cytotoxicity and finally abnormal production of and response to different lymphokines. The goal of these studies is to further elucidate the mechanisms of these alterations of the immune system which are apparently involved in the pathogenesis of this disease. The modulation of the above disturbances by immunosuppressive agents, i.e. corticosteroids and cyclophosphamide, is actively studied aiming at the restoration of normal immune status in these patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 41053-05 ARB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effects of High-Dose Intravenous Methylprednisolone on the Course of RA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: R.L. Wilder, Senior Investigator, ARB, NIADDK

COOPERATING UNITS *(if any)*

CPRL, PDS, Clinical Center

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Diseases

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0		

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

  ☐ (a1) Minors

  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

PART ONE-Six patients with rheumatoid arthritis (RA) unresponsive to conventional therapies have been studied in an open pilot trial of short-term pulse methylprednisolone (MP). Following administration of 1 gram of MP intravenous on 3 consecutive days, dramatic changes in articular index were evident in the first 1-2 weeks. The effect persisted for a variable period of time - 2 weeks to more than 8 weeks. No major side effects were observed. No persistent changes in several immunological parameters were measured. The results provide a preliminary basis to design better therapeutic protocols.

PART TWO-Oral administration of high dose pulse corticosteroid is potentially a more practical method of administering pulse therapy. Drug absorption and patient acceptance, however, of high dose orally administered steroids are not well documented. Thus, we studied plasma MP levels in 3 patients with RA after a single IV or oral administration. Results indicate that orally administered high dose MP is well absorbed (60%) and well tolerated, but therapeutic equivalence of IV versus orally administered MP is not certain.

THIS PROJECT HAS BEEN TERMINATED

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 41054-04 ARB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Effect of a Brief Course of Leukapheresis in the Management of RA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: R.L. Wilder, Senior Investigator, ARB, NIADDK

J.B. Allen Chemist ARB, NIADDK

C. Yarboro, Clinical Nurse, ARB, NIADDK

COOPERATING UNITS *(if any)*

MDI, NIDR; NMRI, NNMC

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Disease

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER
0.5	0.25	0.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The effects of a brief course of leukapheresis were studied in fourteen patients with Rheumatoid Arthritis, unresponsive to conventional therapy. The patients consisted of two groups--those with profoundly depressed or normal peripheral blood mononuclear cell proliferation to soluble antigens. They represented the extremes of our rheumatoid patient population. Six of 7 patients with depressed proliferation clinically improved, whereas 0/7 patients with normal proliferation clinically improved following a brief (six runs over two weeks) course of leukapheresis. This and other immunologic abnormalities tended to normalize in the group which clinically improved but not the clinically nonresponder group. Thus, reversal of depressed immune function appeared to parallel clinical improvement. These studies provide new insights into the pathogenesis of rheumatoid arthritis and more insight into the effects of leukapheresis.

THIS PROJECT HAS BEEN TERMINATED

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 41055 04 ARB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Effect of Plasmapheresis on Systemic Lupus Erythematosus (SLE)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.H. Klippel, Senior Investigator, ARB/NIADDK

COOPERATING UNITS *(if any)*

Dermatology Branch, NCI

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Diseases

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 0	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The therapeutic efficacy of short-term plasmapheresis in SLE will be assessed in a double-blind randomized trial. Patients will receive either 4 liter plasmapheresis or reinfusion plasmapheresis 3 times weekly for a period of 2 weeks. Clinical and laboratory assessments of lupus activity will be done with each apheresis and every week subsequent for 4 weeks. The protocol will be completed at the end of the 6th week or if the patient is withdrawn as a clinical therapeutic failure.

THIS PROJECT HAS BEEN TERMINATED

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 41056-04 ARB

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunosuppressive Drug Therapy in Lupus Glomerulonephritis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

J. E. Balow, (P.I.)	Senior Investigator	ARB, NIADDK
J. H. Klippel	Senior Investigator	ARB, NIADDK
P. A. Plotz	Senior Investigator	ARB, NIADDK
A. D. Steinberg	Senior Investigator	ARB, NIADDK
R. L. Wilder	Senior Investigator	ARB, NIADDK
H. A. Austin	Expert	CC

COOPERATING UNITS *(if any)*

Walter Reed Army Medical Center, Washington, DC

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.2	1.4	0.8

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The efficacy of intensive, intermittent immunosuppressive drug therapy will be evaluated in patients with active lupus glomerulonephritis over a 30 month study period. Patients with renal biopsy documented active glomerulonephritis with or without renal functional deterioration will be treated with low dose corticosteroids and randomized to receive (a) intravenous pulse methylprednisolone monthly for 6 months or (b) intravenous pulse cyclophosphamide monthly for 6 months or (c) intravenous pulse cyclophosphamide monthly for 6 months and then every 3 months for the remaining 24 months of the study. During the final 24 months of the study, all patients will receive low dose prednisone. Active disease, as manifested by renal functional deterioration, increased proteinuria or worsened urinary sediment, will be treated by increased prednisone. Comparison will be made of the number of favorable outcomes of renal function, glomerular pathology and drug related toxicities achieved by each treatment group at the end of the 6th and 30th study months. Between April 1981 and July 1984 there have been more than 35 patients entered into this protocol.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41057-03 ARB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Low Dose Methotrexate Therapy in Rheumatoid Arthritis (RA)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.H. Klippel, Senior Investigator, ARB/NIADDK

COOPERATING UNITS *(if any)*

Rehab, CC.

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Diseases

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study will evaluate the benefits, incidence and severity of side effects of low dose pulse oral methotrexate in patients with rheumatoid arthritis in a double-blind study. Twenty five patients will be randomized to receive either low dose oral methotrexate (7.5-15mg/week) or placebo for 18 weeks. The Arthritis and Rheumatism Branch is one of eleven clinics carrying out this protocol under the direction of the Cooperating Clinics for the Systematic Studies of Rheumatic Diseases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 41059-03 ARB

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunosuppression and Plasmapheresis in Goodpasture's Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

J. E. Balow, Senior Investigator, ARB, NIADDK
H. A. Austin, Expert, C.C.

COOPERATING UNITS *(if any)*

Walter Reed Army Medical Center, Washington, DC
Scripps Clinic and Research Foundation, La Jolla, CA

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.25	.25	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Goodpasture's Syndrome is usually characterized by rapidly progressive glomerulonephritis due to autoantibodies against glomerular basement membrane (anti-GBM). This study will examine whether plasmapheresis significantly improves the results of standard immunosuppressive drug treatment of this disease. Patients will be randomly allocated to drug treatment alone or drugs plus plasmapheresis until anti-GBM levels are undetectable. Comparison will be made of the numbers of favorable outcomes and complication rates of these therapies at the end of 6 months of study.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 41060-02 ARB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Monocyte Fc receptors in systemic lupus erythematosus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. Paul H. Plotz	Senior Investigator	ARB/NIADDK
W. Mullins	Medical Staff Fellow	ARB/NIADDK

COOPERATING UNITS *(if any)*
NIAID

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Disease

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 0.2	PROFESSIONAL: 0.2	OTHER:

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Much evidence suggests that tissue damage in systemic lupus is caused by the deposition of immune complexes in tissues, followed by inflammation. We have in the past shown, in human lupus, a defect in the ability of the reticuloendothelial system (RES) to remove particulate immune complexes (antibody-coated red cells) from the circulation. To determine whether simple overload of receptor for immunoglobulin in the RES might be responsible, capacity of circulatory RES cells - perhipheral blood monocytes - to bind immunoglobulins was measured. Contrary to expectation, the capacity was increased in patients with lupus, and the increase was correlated with disease activity, thus ruling out simple overload as an explanation for the earlier observed RES failure.

THIS PROJECT HAS BEEN TERMINATED

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 41061-02 ARB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Dendritic cells as antigen-presenting cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Paul H. Plotz	Senior Investigator	ARB/NIADDK
	W. Mullins	Med. Staff Fellow	ARB/NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Disease

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Dendritic cells are cells of characteristic shape, density, and surface properties found in small number in lymphoid and other organs. Their importance lies in the fact that they have a heavy representation of Ia molecules on their surface (molecules believed to be important in allowing antigen to be recognized by lymphocytes on the surface of antigen-presenting cells) and that they are extremely potent stimulators to lymphocytes in vitro. Dendritic cells have been isolated in our laboratory from lymph nodes using established techniques. At present, methods are being developed to obtain larger numbers of cells using the spleen so that studies of their surface receptors can be carried out.

THIS PROJECT HAS BEEN TERMINATED

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41062-02 ARB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Handling of immune complexes containing nuclear antigens

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. Paul H. Plotz	Senior Investigator	ARB/NIADDK
L. Schrieber	Visiting Associate	ARB/NIADDK

COOPERATING UNITS *(if any)*
Dept. of Molecular Biology, Yale University

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Disease

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesad, MD 20205

TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 1.0	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previous investigations in this laboratory have shown that, under certain circumstances, antigen structure may critically determine the way in which an immune complex is handled in an animal. It is known that the presence of certain antibodies in patients with systemic lupus or related diseases define clinical categories of patients. With the purification of the antigen against which those antibodies are directed, it has become possible to ask if the structure of the antigen determines the behavior of the complexes. It is the purpose of these studies to explore the metabolism of immune complexes made from the anti-Sm antibodies, which can react with either of two distinct nuclear particles (U1 or U2-6), and to compare the metabolism of complexes of U1 particles with either anti-Sm antibodies or anti-RNP antibodies. So far, small quantities of U2-6 have been radiolabeled and been traced after injection into mice with or without auto-antibodies, either exogenously added or endogenously produced (MRL/l mice).

THIS PROJECT HAS BEEN TERMINATED

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 41063-02 ARB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Severe acute pulmonary disease in systemic lupus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. Paul H. Plotz	Senior Investigator	ARB/NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Disease

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐x(a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Project Terminated

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 41064-02 ARB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Bacterial endocarditis in systemic lupus erythematosus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. Paul H. Plotz	Senior Investigator	ARB/NIADDK
Cheryl Yarboro	Nurse	CC

COOPERATING UNITS *(if any)*
NBLBI
Clinical Center - Nursing Department

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Disease

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Project Terminated

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41065 02 ARB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of the renal disease of systemic lupus erythematosus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Paul H. Plotz	Senior Investigator	ARB/NIADDK
	J.E. Balow	Senior Investigator	ARB/NIADDK
	J.H. Klippel	Senior Investigator	ARB/NIADDK
	A.D. Steinberg	Senior Investigator	ARB/NIADDK
	R.L. Wilder	Senior Investigator	ARB/NIADDK

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Arthritis and Rheumatism Branch

SECTION
Connective Tissue Disease

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 1	PROFESSIONAL: 1	OTHER: Protocol # 82 A 201

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients with systemic lupus erythematosus under the care of NIH will be categorized with 5 categories on the basis of their renal findings. Two groups - those with little or no evidence for renal disease - will be followed systematically. An intermediate group will have kidney biopsy, then be followed systematically and probably be the focus of a therapeutic trial in the near future. A group with serious disease is already the focus of a major therapeutic protocol. Patients with far-advanced disease considered unresponsive to therapy will be referred elsewhere. The major objectives are to collect data systematically on patients with lupus in order to understand the course of illness prior to the development of major renal disease so that it may be prevented.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 41066-02 ARB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunologic Characterization of Synov. Tissue from Patients w/RA & Related Cond.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: R.L. Wilder,	Senior Investigator,	ARB/NIADDK
D.G. Malone,	Medical Staff Fellow,	ARB/NIADDK
J.B. Allen	Chemist	ARB/NIADDK
B. Haraoui	Guest Researcher	ARB/NIADDK

COOPERATING UNITS *(if any)*

Laboratory of Microbial Immunology, NIDR
Laboratory of Pathology, NCI

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Diseases

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Synovial tissues and peripheral blood mononucler cells are obtained from patients with active chronic synovitis, such as rheumatoid arthritis. The analysis of these samples suggests that conditions, such as rheumatoid arthritis, comprise an immunopathologic spectrum. Patients at the polar extremes of this spectrum display consistent phenotypic and functional characteristics. Further analysis of these patients may provide insight into potential etiologic mechanisms or improved therapy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 41067-02 ARB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

In Vitro Study of Normal Human Lymphocytes/Monocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: R.L. Wilder, Senior Investigator, ARB/NIADDK
B. Haraoui, Senior Investigator ARB/NIADDK

COOPERATING UNITS *(if any)*

LML, NIDR; NMRI, NMRC, NNMC

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Diseases

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 0.5	PROFESSIONAL: 0.5	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Normal human lymphocytes and monocytes, obtained by leukapheresis, were separated into distinct fractions by counterflow centrifugal elutriation. Immunologic function and phenotype was studied in vitro for each fraction. Distinct characteristics were associated with each fraction. The studies add to our understanding of mononuclear cell function in normal individuals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41068-01 ARB

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Human Immune Response by Complement

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

G. C. Tsokos, Visiting Scientist, ARB, NIADDK
J. E. Balow, Senior Investigator, ARB, NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	0.8	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Complement partakes in the expression of human immune responses. Complement factors and breakdown products block the differentiation of human B lymphocytes in vitro. Complement components and receptors also affect the generation of cytotoxic T cells in vitro. Understanding of the mechanism of regulation of immune responses by complement is crucial for the understanding of the immunopathogenesis of autoimmune diseases since they are frequently associated with complement activation and depression of complement factor levels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 41069-01 ARB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The role of viruses in the induction of autoantibodies in humans and mice

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. Paul H. Plotz	Senior Investigator	ARB/NIADDK
F. Miller	Medical Staff Fellow	ARB/NIADDK
T. Biswas	Visiting Fellow	ARB/NIADDK
M. Cronin	Medical Staff Fellow	ARB/NIADDK

COOPERATING UNITS *(if any)*
Laboratory of Oral Medicine, NIDR

LAB/BRANCH
Arthritis and Rheumatism

SECTION
Connective Tissue Disease

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Characteristic antibodies which react with normal tissues (autoantibodies) develop in patients with a number of diseases and in some animals. Considerable evidence suggests that some of these antibodies arise because of specific interactions between an exogenous agent (virus, bacterium, drug) and the sites to which the antibody binds. A search is underway to determine whether certain picornaviridiae can be implicated in the causation of inflammatory myositis and the characteristic myositis related antibodies which react with amino acid t-RNA synthetases.

ANNUAL REPORT OF THE METABOLIC DISEASES BRANCH
National Institute of Arthritis,
Diabetes, and Digestive and Kidney Diseases

The general goals of the Branch are to investigate the structure, secretion and biochemical mechanism of action of hormones governing ion transport and mineral metabolism and to apply the knowledge gained to understanding and treating related clinical disorders. Current investigations include hormone-receptor interaction, mechanism of receptor and guanine nucleotide control of adenylate cyclase, cyclic AMP control of ion transport, regulation of parathyroid hormone secretion, and regulation of response to for biologically active vitamin D metabolites. Systems under study include -adrenergic receptors in several tissues, isolated parathyroid cell systems and skeletal and renal tissue. Clinical studies on hyperparathyroidism and hormone-resistant states continue to utilize extensively the research findings from the laboratory. 1,25-dihydroxyvitamin D (1,25(OH)$_2$D) receptor defects have been identified in skin fibroblasts from cases of osteomalacia resistant to 1,25(OH)$_2$D. A deficiency of the guanine nucleotide regulatory protein has been discovered in many cases of pseudohypoparathyroidism. Radioimmunoassays for parathyroid hormone have been applied to determine the hormone in samples obtained selectively by venous catheterization or by fine needle aspiration, thereby allowing localization of parathyroid adenomas. Further studies make use of urinary cyclic AMP clearance as a clinical parameter of parathyroid hormone action. Another investigation takes advantage of the autologous parathyroid transplant program developed in collaboration with the Surgery Branch, NCI. Autologous transplants to the arm allow direct sampling of draining veins for parathyroid hormone secretion in man under conditions wherein the tissue can be selectively challenged with potential secretagogues or inhibitors of parathyroid secretion.

Analysis of Hormone Receptor.

Interactions of catecholamines with adrenergic receptors and activation of adenylate cyclase are under study with the plasma membranes of several cell systems. Specific receptors have now been identified on turkey erythrocytes, parathyroid cells, pineal cells, rat, guinea pig and monkey lung membrane preparations, rat osteosarcoma cells and rat liver membranes. Control of receptor accumulation in isolated cell culture systems in vitro are being studied with a view toward gaining knowledge about the molecular biology of receptors.

Receptors for Calcitonin and Parathyroid Hormone

Studies in collaboration with Dr. T. Murray (St. Michael's Hospital, Toronto) have led to development of radiolabeled intact bovine parathyroid hormone as a ligand. This material binds to receptors in canine renal plasma membranes or in cultured osteosarcoma cells with kinetic properties distinct from those of radiolabeled synthetic amino terminal bioactive fragments. Thus the new ligand should prove useful for evaluating PTH-receptor interactions of the native hormone. (Drs Marx, Aurbach, and Pizzoli).

Binding of radioiodinated parathyroid hormone was successfully analyzed on specific receptors on cultured rat osteosarcoma cells. Binding to receptors correlated well with stimulation of cyclic AMP production in this cell system.

Guanine Nucleotide Binding Proteins

Studies on the structure and function of guanine nucleotide binding proteins (G proteins) are continuing. Two distinct proteins, G_s and G_i, are now known to couple stimulatory and inhibitory hormone receptors to adenylate cyclase. G_s is a substrate for ADP-ribosylation by cholera toxin and G_i is similarly modified by pertussis toxin. A G-protein located in retinal rod outer-segment membranes termed transducin has been found to be functionally homologous to G_s and G_i. Transducin couples rhodopsin to a cyclic GMP phosphodiesterase. We have found transducin to be a substrate for both cholera and pertussis toxins. This suggests structural in addition to functional homology for this family of G proteins. Because of this homology, and because transducin is more readily purified than G_s and G_i, we developed methods for purification of milligram quantities of transducin. We immunized rabbits and mice with purified holoprotein and developed an Elisa to screen sera for antibodies. Several rabbits developed high-titer antibodies to transducin. Immunoblot analysis of SDS-PAGE separated subunits of transducin showed that the sera varied in their reactivity with individual subunits. This should enable us to probe the functions of the individual subunits with specific antisera. Antisera that recognize the beta (35k) subunit of transducin were found (by immunoblot analysis of SDS-PAGE separated membrane proteins) to cross-react with a single plasma membrane peptide (35k) from non-retinal tissues, including bovine liver, kidney and brain, S49 mouse lymphoma cells (including the CYC- mutant), turkey erythrocytes, and chick retina, pituitary and pineal. Brain showed the highest amount of this peptide.

Immunoblot analysis of the purified subunits of G_s and G_i (from human erythrocytes, provided by L. Birnbaumer, Houston) with antisera against all three transducin subunits showed cross-reactivity of the beta subunits, but not of the alpha or gamma subunits. This suggests that only the beta subunits are very similar in structure, and raises the possibility that the gamma subunit confers specificity (? in interactions with receptors or with alpha subunits) to the holoprotein. These experiments also confirm the identity of the peptide detected on immunostaining of plasma membranes as the common beta subunit of G_s and G_i. The antisera should prove useful in immunoaffinity purification of G_s and G_i, in immunocytochemical studies, in probing the role of the beta subunit in intact membranes, and in screening expression vector libraries for the genes for transducin subunits, and for the common beta subunit of G_s and G_i. Experiments to investigate these possibilities are in progress.

Work is also in progress on production of monoclonal antibodies to transducin. Sera from mice immunized with transducin were shown to contain antibodies by Elisa. 3 successful fusions have been performed up to now. Elisa-positive hybridomas have been expanded and cloned, and large volumes of supernate have been produced. Antibodies are being characterized in terms of heavy chain class, transducin subunit specificity, and cross-reactivity with the homologous G proteins G_s and G_i. Monoclonals should yield valuable comparative structural information on the three G proteins, as well as insights into the functions of the individual subunits.

Studies on reconstitution of purified receptors, G proteins, and adenylate cyclase into phospholipid vesicles are being performed in collaboration with the labs of R. Lefkowitz (Duke) and L. Birnbaumer (Houston). Thus far, we have shown that purified beta receptors couple with all three G proteins but that agonist-stimulated GTPase activity is greatest for G_s, with G_i and transducin showing 10% or less activity. In contrast, purified rhodopsin (light-activated) stimulates transducin and G_i much more effectively than G_s. Since purified receptors of the inhibitory class e.g. alpha 2, are not yet available in purified form, rhodopsin's ability to couple with G_i can be used to study the interaction between G_s and G_i in reconstituted systems. Preliminary results show that rhodopsin stimulated G_i inhibits beta adrenergic receptor stimulated (via G_s) adenylate cyclase activity. [Drs. Spiegel and Gierschik, Mrs. Simons, NIADDK: L. Birnbaumer and J. Codina, Houston; R. Lefkowitz and P. Cerioni, Duke; R. Somers, Lab of Vision Research, NEI).

Pseudohypoparathyroidism (PHP)

Previous studies identified the molecular defect responsible for hormone resistance in some patients with PHP as a deficiency in G_s activity. Such patients show the phenotypic features of Albright's hereditary osteodystrophy (AHO) and resistance to multiple hormones. Adenylate cyclase activity is now known to be regulated by a stimulatory, G_s, as well as an inhibitory, G_i, G protein. Both G proteins share a common beta subunit, and it has been suggested that G_i acts to inhibit G_s through this common beta subunit. Thus apparent G_s deficiency could result from a number of specific changes in either G_s or G_i. We have directly measured the alpha subunit of G_i (by radiolabeling with pertussis toxin) in red cell membranes of patients with various forms of PHP, and found normal amounts (in contrast with the reduced amount of alpha subunit of G_s labeled with cholera toxin). With the availability of antibodies that specifically recognize the beta subunit of G_s and G_i (see G proteins, above), we plan to quantitate the amount of beta subunit in membranes from patients with various forms of PHP.

Patients with PHP without AHO generally show normal G_s activity, and resistance limited to PTH. We have found that cultured skin fibroblasts respond to PTH (as well as prostaglandins and forskolin) with a rise in cAMP production. We have now established multiple fibroblast lines from patients with PHP (skin biopsies from several patients were kindly provided by Dr. Arnold Moses, Syracuse, and Dr. Neil Breslau, Dallas). Preliminary results show that fibroblasts from some patients are deficient in PTH responsiveness, but show normal prostaglandin and forskolin responsiveness. These data suggest a PTH receptor defect, which in certain familial cases, could be inherited. Further studies to elucidate a possible PTH receptor defect, including use of a specific PTH receptor photoaffinity reagent, are planned. [Drs. Spiegel and Silve, NIADDK; Dr. Downs, Med Coll Va.]

Primary Hyperparathyroidism and Familial Hypercalcemia

Clinical studies are continuing on primary hyperparathyroidism and its familial variants. Detailed family screening and case finding has produced 52 kindreds for analysis. These studies have allowed segregation of the familial variants into two apparently distinct disease syndromes

- familial multiple endocrine adenomatosis (MEN I) and familial hypocalciuric hypercalcemia (FHH). FHH was distinguished from MEN I by 1) virtually a 100% penetrance for hypercalcemia before age 20, 2) milder clinical manifestations - low incidence of recurrent nephrolithiasis or recurrent peptic ulceration, 3) no hypercalciuria, 4) normal basal concentrations of gastrin, and 5) poor response to subtotal parathyroidectomy. Distinction between the two syndromes, both inherited as autosomal dominant traits, is important because in FHH the clinical course is generally milder and subtotal parathyroidectomy is less likely to be beneficial. Para-thyroidectomy is frequently unsuccessful in FMENI if the multiglandular nature of the disorder is not recognized intraoperatively. FHH accounts for approximately 10% of all unsuccessful parathyroidectomies in hypercalcemia. In FHH the ionized and ultrafiltrable calcium concentration in serum is elevated in proportion to the increase in total calcium. In these patients the filtrable load of calcium is high in association with a marked decrease in renal calcium clearance. The concentration of parathyroid hormone in plasma is lower in patients with FHH than in typical primary hyperparathyroid patients with similar degrees of hypercalcemia whether assessed by PTH radioimmunoassay or by renal clearance of cAMP or phosphate. The parathyroid glands show hyperplasia in most cases. In several kindreds one or more members have exhibited life-threatening primary hyperparathyroidism in the neonatal period. This may result sometimes from a double dose of the FHH gene. The plasma and urine cAMP response to PTH infusion is greater in FHH than in typical primary hyperparathyroidism. Even when these patients become surgically hypoparathyroid, the low renal clearance of calcium is strikingly persistent during calcium infusion. (Drs Marx, Spiegel, Fitzpatrick, and Aurbach).

Studies on modes of localizing parathyroid tumors continue. A high degree of success has been obtained in localizing tumors through vascular catheterization procedures. Parathyroid arteriography developed and performed by Dr. John Doppman afforded, in approximately 45% of cases tested, the identification of abnormal masses of tissue subsequently identified as parathyroid. Confirmation of the mass thus identified as parathyroid tissue can be accomplished by verifying high concentrations of parathyroid hormone by radioimmunoassay in veins draining the lesion. Draining vessels contain concentrations of hormone 2- to 20-fold or more greater than the concentration of parathyroid hormone detected by immunoassay in the peripheral plasma. In cases with single adenomas, this concentration ratio is greatest selectively in the one vein draining the lesion. This technique utilizing specific radioimmunoassays and selective catheterization is applicable also to determining the source of hormone in ectopic hyper-parathyroidism produced by non-parathyroid cancers. Tentative preoperative identification of a parathyroid mass can be confirmed by fine needle aspiration. We have aspirated with guidance by comupterized tomography or ultrasound approximately 20 such lesions that were subsequently confimred surgically as parathyroid. RIA of the aspirates showed high concentrations of PTH in all but one.

Localization of anterior mediastinal and cervical-paratracheal parathyroid adenomas has become possible using computed tomography. Eight mediastinal adenomas have been treated nonsurgically by percutaneous injection via catheter of occlusive agents into the arterial blood supply with 7 complete and one partial remissions. (Drs Aurbach, Marx, Spiegel, Fitzpatrick, Santora, NIADDK; Dr Norton, NCI, Drs Doppman and Krudy Diagnostic Radiology, CC).

A new series of radioligands for parathyroid hormone radioimmunoassay has been developed, using synthetic sequences and analogs from the midregion of human PTH. These reagents have led to development of sensitive and practical radioimmunoassays. Using these assays and gel filtration of plasmas, we have demonstrated that certain hyperfunctioning parathyroid glands release a previously unrecognized large PTH fragment containing the midregion but deficient in carboxyterminal immunoreactivity. (Dr Marx, Mr Sharp).

Rapid determination of intraoperative UcAMP excretion (using the Gammaflo machine for 7 min cAMP radioimmunoassay) has proven to be a valuable tool in guiding surgery for primary hyperparathyroidism. Persistent elevation of UcAMP requires continued search for abnormal tissue even after 1 or more abnormal glands have been removed. A rapid (mean 1.5 hours) drop in UcAMP to the normal range obviates the need for continued exploration even in cases where histologic confirmation of parathyroidectomy is lacking. Spurts in UcAMP above baseline may provide a clue to the location of abnormal parathyroid tissue. (Drs. Spiegel, Marx, Downs and Aurbach, NIADDK: Dr. Norton, NCI Surgery).

Determination of urinary cAMP excretion postoperatively in patients undergoing neck exploration for primary hyperparathyroidism is a useful method for assessing postoperative parathyroid function. UcAMP excretion declines postoperatively in all patients in whom hypercalcemia is corrected but not in those with persistent hypercalcemia. In patients becoming severely hypocalcemic (and requiring vitamin D therapy) postoperatively, UcAMP measurement enables one to distinguish patients with decreased parathyroid reserve as the cause for hypocalcemia (low UcAMP excretion) from patients with healing osteitis fibrosa ("hungry bones" with secondary hyperparathyroidism) as the basis for hypocalcemia. UcAMP in the latter group is often elevated but can be suppressed if serum calcium is normalized. Elevated UcAMP excretion postoperatively in the face of hypocalcemia enables one to predict that vitamin D therapy will be required temporarily and precludes the need for parathyroid autografts. (Drs. Spiegel, Marx, Downs, Levine, and Aurbach, NIADDK).

Secretion of Parathyroid Hormone

Studies have continued with dispersed bovine and human parathyroid cells. PTH secretion from parathyroid glands in vivo and cells in vitro is inhibited by calcium. Calcium may also modulate synthesis and degradation of PTH. This control exerted by calcium may be changed in certain pathologic states (glandular adenomas, carcinomas and perhaps hyperplasia). Several earlier studies have strongly suggested that cAMP is an intracellular mediator of PTH secretion; agents which alter cellular cAMP change PTH secretion in the same direction. Calcium decreases cellular cAMP, but most of its effect to inhibit secretion is independent of changes in cellular cAMP.

Inhibition of cAMP accumulation is being studied in isolated parathyroid cells. Inhibitory effects of alpha-adrenergic and PGF_{2alpha} are found in normal but not abnormal glands. These differences may reflect an abnormal control mechanism governing secretion in hyperparathyroidism. (Drs. Aurbach, Fitzpatrick, Brown).

We have discovered several novel peptides in extracts of stomach tissue from pigs that stimulate cAMP accumulation and parathyroid hormone release from parathyroid cells in vitro. Two peptides are related to VIP and secretin, respectively. Two are completely unknown. We have named the latter parathyroliberins (PTL) alpha and beta. PTL alpha stimulates secretion from human parathyroid cells and PTL beta from bovine cells. Isolation of these peptides should permit development of radioimmunoassays and determination of their physiologic significance (Drs. Aurbach, Brown, McDonald, and Mutt).

Humoral Hypercalcemia of Malignancy

A Leydig cell transplantable tumor causes hypercalcemia in rats. cAMP is increased in the urine of the tumor-bearing animals but plasma PTH is undetectable. Thus hypercalcemia apparently can be attributed to a non-parathyroid tumor factor that may, like parathyroid hormone, act through the adenylate cyclase-cyclic AMP system. This humoral factor, whose identity we wish to establish, presumably causes hypercalcemia by resorption of bone. Tumor cells cultured in vitro elaborate into the medium, a bone resorbing substance. Histomorphometric studies of bone from tumor-bearing and control animals show that the tumor factor causes extensive osteoclastic bone resorption and results in a major loss in trabecular bone. In addition, tetracycline labeling studies demonstrate a marked decrease in bone formation in tumor-bearing rats.

Large amounts of conditioned medium (CM) from cultured leydig tumor cells have been prepared for purification. CM is concentrated and desalted and extracted with guanidine. Purification steps include gel filtration, reverse phase c-18 HPLC, and ion exchange HPLC. CM infused into thyroparathyroidectomized rats stimulated urinary cAMP excretion (as in tumor-bearing rats) and maintained serum calcium. CM was found to stimulate cAMP production in an osteoblast-like rat cell line, ROS 17/2.8, as well as in human skin fibroblasts. The former activity is being used to monitor purification of CM. The latter activity suggests that the leydig tumor factor resembles factor(s) elaborated by human tumors associated with the syndrome of humoral hypercalcemia. (Drs. Spiegel, Santora, Silve, NIADDK; Dr. Norton, NCI)

Vitamin D Resistance and Related Disorders

The role of 1,25(OH)$_2$D3, the most potent natural metabolite of vitamin D, has been assessed in hypocalcemic states. This very rapidly acting drug has simplified the management of hypocalcemia following parathyroidectomy; during this time skeletal remineralization imposes large but rapidly diminishing requirements for calcium.

We have evaluated patients with extreme resistance to 1,25(OH)$_2$D. This can be a transient state as following parathyroidectomy or a permanent state as in familial cases. We have evaluated ten patients with familial resistance to 1,25(OH)$_2$D. Most patients have hypocalcemic rickets, usually with associated total alopecia. Mineral homeostasis is usually improved by treatments that sustain 1,25(OH)$_2$D levels at 10-100 times normal.

Specific intracellular defects have been evaluated using cultured skin fibroblasts from these patients. With skin fibroblasts cultured from normals, a typical 1,25(OH)$_2$D-receptor can be identified by binding in soluble extracts, by nuclear uptake of hormone with intact cells, or by density gradient analysis of labeled hormone in extracts of cytosol or nucleus.

Fibroblasts from patients with familial resistance to 1,25(OH)$_2$D have shown a spectrum of defects including nonfunctional receptors, diminished numbers of receptors, and apparently normal receptors. In one patient, osteoblast-like cells from bone biopsy exhibited a defect analogous to that in skin fibroblasts of the same patient. Even when receptors have unmeasurable hormone-binding activity, the receptor protein has been present in normal amounts according to immunoassay. Cellular action of 1,25(OH)$_2$D3 can be analyzed by measuring its induction of the 25(OH)D 24-hydroxylase enzyme system. Cultured skin fibroblasts from patients with hereditary resistance to 1,25(OH)$_2$D exhibit defects in this inductio even when nuclear receptors are normal by hormone binding assays. The association of alopecia with multiple distinct genetic mutations in the 1,25(OH)$_2$D effector system indicates that 1,25(OH)$_2$D-resistance is one direct cause of total alopecia. Drs. Marx and Liberman, MDB, NIADDK; Drs. Eil and Gamblin, BNMC; Dr. Balsan, Paris; Dr. Rosen, Albert Einstein; Drs. Pike and Haussler, U. Arizona).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 43002-19 MD

PERIOD COVERED
October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure, Secretion and Mechanism of Action of Parathyroid Hormone

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
G.D. Aurbach, M.D., Chief, MDB, NIADDK

COOPERATING UNITS *(if any)*
Endocrine Unit, Massachusetts General Hospital

LAB/BRANCH
Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS: 3.00	PROFESSIONAL: 1.00	OTHER: .25

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

There is still much to be learned about the nature of circulating parathyroid hormone in clinical disease. It is the purpose of this project to study the function and mechanism of action of parathyroid hormone, study its relationship to human disease, and develop clinically useful tests for circulating parathyroid hormone. From these studies it is expected that one can understand the pathophysiology of certain metabolic diseases of bone and endocrine disturbances. The entire structures of bovine, porcine and human parathyroid hormone have been determined. Synthetic polypeptides representing the first 34 residues of bovine and human parathyroid hormone have been prepared. These molecules show all the biological properties of the native hormonal polypeptide. Highly sensitive radioimmunoassays for the hormone have been developed and are being modified further for improved clinical diagnostic parameters. Studies show that the mechanism of action of the hormone is mediated through direct hormonal activation of adenylate cyclase in bone and kidney. An isolated parathyroid cell system has been developed that allows studies of secretagogues for parathyroid hormone.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 43003-19 MD

PERIOD COVERED
October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on the Mode of Action of Thyrocalcitonin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
G. D. Aurbach, M.D., Chief, Metabolic Diseases Branch, NIADDK

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Md. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose is to study the interaction of calcitonin with its specific receptor target organs. The current investigations should provide further insight into the structure-function relationship in calcitonin. Calcitonin is a small polypeptide hormone and therefore lends itself well to studies using synthetic peptide fragments. The system is also useful for characterizing hormone receptors in kidney, bone and other tissues. Studies are in progress to characterize further the interaction of calcitonin with tissue receptors. It also will be of interest to solubilize the receptors and characterize them chemically.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 43004-19 MD

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on pseudohypoparathyroidism and related disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

G. D. Aurbach, M.D. Chief, Metabolic Diseases Branch, NIADDK

COOPERATING UNITS *(if any)*

NICHD Developmental Endocrinology Branch (CRF and GRF tests).

LAB/BRANCH

Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Md. 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.50	.50	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In 1942 Albright and his associates described the features of a new clinical syndrome "pseudohypoparathyroidism" (PHP). Patients with this disorder differ from those with idiopathic hypoparathyroidism: they show characteristic constitutional features (Albright's hereditary osteodystrophy - AHO) and do not respond to exogenous parathyroid hormone. Subsequent to the original report, patients lacking the typical somatic features of AHO but resistant to endogenous and administered PTH have been described. We found previously that in PHP, U_{cAMP} (urinary cyclic AMP) does not increase normally in response to PTH administration. This indicated that there is a defective hormone receptor-adenylate cyclase complex in this disorder. We have now shown that many patients with PHP+AHO show an approximately 50% reduction in G_s (the stimulatory receptor adenylate cyclase coupling protein) in membranes from multiple tissues. G_s deficiency presumably accounts for resistance to multiple hormones in such patients. Most patients with PHP without AHO show normal G_s activity and preliminary studies suggest a PTH receptor defect in such patients.

PHS 6040 (Rev. 1/84)

GPO 904-917

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 43005-19 MD

PERIOD COVERED
October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Guanine nucleotide binding proteins as receptor-effector couplers.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: A. M. Spiegel

COOPERATING UNITS *(if any)*
Dept. of Cell Biology, Baylor University, Houston;
Howard Hughes Research Lab, Duke University, Durham;
Laboratory of Vision Research, NEI.

LAB/BRANCH
Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, N.I.H. Bethesda, Md.

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.00	2.00	1.00

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Guanine nucleotide binding proteins, G proteins, function as receptor effector couplers. Two distinct G proteins, G_s and G_i, couple stimulatory and inhibitory hormone receptors respectively to adenylate cyclase. A homologous retinal protein, transducin, couples a light receptor, rhodopsin, to cGMP phosphodiesterase. We have purified transducin, raised antibodies to it, and used these to compare the structure and function of G_s, G_i, and transducin. These studies provide important insights into the mechanism of signal transduction across the cell membrane.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 43006-09 MD

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study of Hyperparathyroidism: Etiology, diagnosis and treatment

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

G. D. Aurbach, M.D., Chief, Metabolic Diseases Branch, NIADDK

COOPERATING UNITS *(if any)*

Radiology Department, CC; Surgery Branch, NCI; Department of Biochemistry, University of Wisconsin; Endocrine Unit, MGH; Digestive Diseases Branch, NIADDK

LAB/BRANCH

Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS: 3.00	PROFESSIONAL: 1.50	OTHER: 1.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The project goal is the evaluation and treatment of hyperparathyroidism. Patients with persistent postoperative hyperparathyroidism are referred for evaluation and treatment. Hereditary hyperparathyroidism in particular is under investigation in the hopes of delineating hereditary molecular abnormalities in glandular regulation, as exemplified in the multiple endocrine neoplasia syndromes. Evaluation ranges from epidemiologic studies of families to in-house studies of patients and to in vitro analyses of excised tissue. Techniques currently being employed and improved include radioimmunoassay of parathyroid hormone, selective arteriography and selective venous sampling for parathyroid hormone, parathyroid gland cryopreservation and autotransplantation, and transcatheter parathyroid gland infarction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 43007-04 MD

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study of humoral hypercalcemia of malignancy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Allen M. Spiegel, M.D. Senior Investigator, MDB, NIADDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.00	.75	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Hypercalcemia is a common and serious complication of cancer. In some patients, hypercalcemia occurs without direct tumor invasion of bone, presumably via a humoral mechanism. The identity of humoral factor(s) is not known, although PTH or prostaglandins may be the cause in some cases. The project goal is to identify factors responsible for humoral hypercalcemia of malignancy. This involves study of patients with this syndrome as well as an animal model for the syndrome, a Leydig cell tumor of Fischer rats.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 43008-03 MD

PERIOD COVERED
October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Vitamin D Resistance and Related Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
S. J. Marx, M.D., Senior Investigator, MDB, NIADDK

COOPERATING UNITS *(if any)*
Endocrine Section, USUHS, Bethesda
Biochemistry Dept., Univ. of Arizona, Tucson
Metabolism Section, Albert Einstein Med School, Health Science Center

LAB/BRANCH
Metabolic Diseases Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.00	.50	.75

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In 1937 Albright and co-workers suggested that rickets or osteomalacia might result from deficient action of vitamin D. With recognition that vitamin D is the precursor to 1,25(OH)2D, a steriodal hormone, it has become possible to identify patients with defective activation of vitamin D and others with defective target action of 1,25(OH)2D. We have shown that patients with hereditary resistance to 1,25(OH)2 exhibit a spectrum of defects in interaction of 1,25(OH)2D with cultured skin fibroblasts. Skin fibroblasts provide a useful model for binding and action of 1,25(OH)2D in its classical target tissues (duodenal mucosa, etc.).

Annual Report of the Clinical Endocrinology Branch, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases

An organizational change was made in CEB during the past year with the creation of the Molecular and Cellular Regulation Section with Bruce Weintraub as Chief. The name of the Endocrine Physiology Section was changed to the Hormone Metabolism and Action Section, and personnel were redistributed between the sections.

Visiting scientists in the Branch came from Belgium, India, Italy, Portugal and Sweden and participated in the Branch's work on the biochemistry and cell biology of endocrine systems and diseases.

Dr. Jacob Robbins received the 1984 Distinguished Service Award of the American Thyroid Association at its annual meeting.

I. Thyroid Biochemistry and Pathophysiology

A. Thyroxine-Protein Interactions

Studies have continued on a new thyroxine-binding protein obtained from human plasma and described in previous annual reports. The 27 K protein, originally found as a contaminant of thyroxine-binding globulin (TBG) preparations, has been further characterized and its biosynthesis investigated. It has now been shown that this protein is immunochemically distinct from TBG and that its biosynthesis in human hepatoma cultured cells is directed by messenger RNA that is separable from TBG mRNA. On the other hand, its ability to bind thyroxine has been confirmed by several techniques, including equilibrium dialysis, from which an association constant of 10^7 M was derived. This is three orders of magnitude lower than the binding constant for TBG. The possible significance of this new protein in thyroid hormone transport is under investigation. (Robbins, Grimaldi, Bartalena)

In a continuation of studies on the molecular properties of TBG, the major T_4 transport protein of human plasma, the possible effect of the oligosaccharide chains on the protein structure has been investigated. Removal of 85 percent of the carbohydrate by treatment with mixed glycosidases caused a decrease in tryptophan fluorescence and a shift in the emission spectrum. It was also found that thyroxine-binding inhibits the changes caused by deglycosylation, thus providing further evidence that occupancy of the T_4-binding site stabilizes TBG structure. The deglycosylated protein, however, was shown to be less stable than native TBG when exposed to mild acid or low concentrations of guanidinium chloride. These studies, contrary to previous theory concerning glycoproteins, indicate that the carbohydrate units can affect the arrangement of amino acids in the apoprotein portion of the molecule. (Robbins, Edelhoch, Grimaldi, Bartalena)

B. Thyroid Hormone Metabolism

The first step in thyroid hormone action is its entry into cells. Until recently this was thought to occur by passive diffusion only, but current work suggests that hepatocytes, fibroblasts and pituitary cells accumulate the hormone by an energy-dependent, saturable mechanism. We are investigating thyroid hormone entry into intact skeletal muscle by using the isolated soleus muscle of rats. About one-third of the accumulation of 3,5,3'-triiodothyronine (T_3) by this tissue can be inhibited by high T_3 levels (above 10^{-8} M) and can be blocked by inhibitors of oxidative metabolism (such as oligomycin) or receptor-mediated endocytosis (such as dansylcadaverine). Thyroxine is a less effective inhibitor than T_3. These findings indicate that skeletal muscle, the largest thyroid hormone sensitive organ, accumulates a significant portion of the intracellular T_3 by a specific uptake mechanism. Two other experimental systems - cultured human hepatoma cells and cultured rat myoblasts - are now under study and preliminary data indicate that a portion of the T_3 entry into these cells is by a saturable energy-dependent process. This is a potential site for regulation of thyroid hormone action. (Robbins, Pontecorvi, Movius, Phyillaier)

To understand more fully the interaction of thyroid hormone with cell membranes, studies with artifical phospholipid membranes are being done. Unilamellar vesicles of dipalmitoyl phosphatidylcholine in which the fluorescent indicator, pyrene, was incorporated, were used to measure the rate of incorporation of T_4 and T_3 at different temperatures. Both hormones bind rapidly when the lipid is in the liquid crystal phase (45° C), but T_4 bound more rapidly than T_3 to the gel phase (35° C). This is probably related to the greater lipophilicity of T_4 compared to T_3. (Movius, Robbins)

C. Thyroid Hormone Action

A major site for thyroid hormone action is at the cell nucleus. Previous work has shown the presence of a saturable T_3 receptor, the occupancy of which is followed by an increase in messenger RNA production for certain proteins. Knowledge of the structure of the receptor protein and its manner of interaction with DNA are still incomplete. In continuation of work on photoaffinity labeling of the receptor using a method that employs underivatized thyroxine, experiments were done to clarify the high background of non-displaceable thyroxine that was encountered. To verify that labeling of the receptor was specific, the method was tested with ten different proteins. Only the two known to have thyroxine-binding sites showed photolabeling. Therefore, it can be concluded that the previously observed 45 kDa and 56 kDa nuclear proteins are specific receptor proteins. (Cahnmann, Nikodem, Magnuson)

One of the proteins responding to thyroid hormone with an increase in cytosolic mRNA is malic enzyme in rat liver. As part of a project to investigate how T_3 modulates the transcriptional activity of the malic enzyme gene, the previously prepared complementary DNA (cDNA) was used as a probe to screen a rat genomic library in bacteriophage Charon 4A, obtained from James Bonner. Two other cDNA probes for T_3-responsive liver proteins are also being used. The work in progress has shown that ten genes interact with malic enzyme cDNA, and eight have been grown in large quantity. These appear to share a common region as defined by restriction analysis and Southern blot analysis when probing with ^{32}P-nick translated malic enzyme cDNA. The goal is to search for sequences that may play a role in T_3 regulation. (Nikodem, Magnuson, Tecce)

A plasmid cDNA library was constructed, representing livers of rats treated with T_3 and a high carbohydrate diet. Using cDNAs to eight different T_3 responsive RNAs, and two T_3-nonresponsive RNAs, the ratio of hybridizable mRNA levels in hyperthyroid compared to the hypothyroid state was determined. These ranged from 0.6 to 30.8. The relation between T_3 nuclear receptor occupancy and hybridizable mRNA levels in hyperthyroid or hypothyroid states compared to normal, showed both linear and non-linear responses for the different mRNAs. In a study of the response kinetics to a receptor saturating dose of T_3 in hypothyroid animals, changes were seen within 4 hr, the earliest time examined, for 6 of the RNAs. For three RNAs, a multiphasic induction was seen, with an early increase and a later decline, followed by a second increase. There also were diverse effects of T_3, diet and growth hormone on the different mRNAs. (Nikodem, Magnuson, Dozin)

Hybridization assays with malic enzyme cDNA have been employed in several studies. It was shown that there are two distinct and functional cytoplasmic mRNAs for this enzyme and that they both increase 10 to 20 fold in hyperthyroidism. Malic enzyme mRNA was assayed in nonhepatic tissues, and was found to increase after T_3 administration in heart and kidney but to be unaffected in brain, lung, testis and spleen. These data provide direct evidence for pretranslational regulation by T_3 of two different mRNAs for malic enzyme and for tissue specificity of hormone control. (Nikodem, Magnuson, Dozin)

D. Studies in Thyroid Disease

In the follow-up of thyroidectomized patients with thyroid carcinoma, we discontinue thyroid hormone at intervals of 6 months to 2 years in order to perform whole body ^{131}I scans. Some of these patients have been enrolled in a study of the effect of short-term hypothyroidism on glucose metabolism in the brain. Although hypothyroidism is known to produce a decrease in total body glucose metabolism as evidenced by lowered BMR, its effect on cerebral glucose metabolism is less well understood. Early studies of total brain oxygen consumption in hypothyroid patients by the nitrous oxide technique of Kety and Schmidt (J.C.I. 27: 476, 1948) suggested a decrease in total brain energy metabolism (Scheinberg, P., et al., J.C.I. 29: 1139, 1950). However, similar studies by Sensenbach et al. (J.C.I. 33:1434, 1954) failed to confirm this decrease. Positron emission tomography (PET) now permits determination of regional glucose metabolism in man. We have used PET to study the effects of acquired hypothyroidism on glucose metabolism in 49 brain regions. (The pituitary and hypothalamus can not be imaged with PET.) Five patients after total thyroidectomy and ablative ^{131}I therapy for papillary carcinoma underwent [^{18}F]-fluorodeoxyglucose PET scans in both the euthyroid and hypothyroid (off T_4 for seven weeks) states. Although glucose metabolism in the total brain and 48 of the areas studied were not significantly changed in the two groups, there was a significant decrease in the regional glucose metabolism in both right and left thalamic regions in the hypothyroid patients. From this study we conclude that cerebral glucose metabolism is regulated by thyroid hormone only in discrete regions and that the depressed affect seen in many hypothyroid patients may be due to an inadequate relay of sensory input by the thalamic nuclei. (Movius, Robbins)

Studies have continued on the syndrome of inappropriate TSH production. During the past year, 4 patients with pituitary neoplasms were investigated. In a 23 year-old woman, the diagnosis of a TSH secreting microadenoma was made on the basis of an elevation of serum TSH and the alpha subunit in blood even though anatomical studies, including computerized axial tomography, were negative. Petrosal sinus sampling suggested localization to the left half of the pituitary, where subsequent transphenoidal surgery demonstrated an 8 mm adenoma. This is the first case of TSH-secreting adenoma to be diagnosed at such an early stage, and surgical cure is to be expected. Two other patients with more typical, long-standing and invasive tumors were investigated. Prior surgery, radiation therapy and drug therapy had failed, both had visual loss and one had concomitant acromegaly. Attempts at further surgical therapy were unsuccessful and one suffered intraoperative death.

Non-neoplastic inappropriate TSH production (thyroid hormone resistance) was investigated in about 40 patients including several affected families. Some were found to have isolated pituitary resistance or generalized resistance to thyroid hormone action. Case-finding in affected families and response to therapy with high dose thyroid hormone was the major emphasis during the past year. Children with the disorder responded to therapy with improved growth and behavior. (Weintraub, Petrick, Menezes-Ferreira)

Patients with central hypothyroidism appear to secrete TSH forms with low biological activity. The basis of this phenomenon and its response to therapy was investigated in patients and in model studies in rats. In patients, serum TSH was purified and the low TSH bioactivity was shown to be due to impaired binding to receptors on human thyroid membranes. A therapeutic trial with chronic TRH injection corrected the receptor binding abnormality and appeared to restore euthyroidism. In the rat studies, pituitary glands from normal and thyroidectomized rats that had been treated with TRH were incubated in vitro and the secreted TSH was examined for biological and immunological activity. By comparison with normal pituitary TSH, thyroid hormone deficiency caused selective secretion of TSH with increased bioactivity. TRH administration enhanced the bioactivity of TSH in normal rats. It is postulated that these alterations are related to changed in TSH glycosylation. (Weintraub, Menezes-Ferreira, Constant)

II. Mechanisms of Cell Secretion

The importance of the cytoskeleton for membrane function and cell secretion has been the subject of further investigation. Tubulin exists in cells in several different forms: a soluble dimer, a polymer in equilibrium with the dimer, and a membrane bound form. In cultured adrenal tumor cells (Y-1) steroidogenesis is stimulated by tubulin-binding drugs (such as colchicine) that inhibit polymerization. In order to test the role of membrane-bound tubulin, a high-molecular weight, carbohydrate-linked derivative of colchcine was synthesized. This molecule should remain external to the cell but the colchicine moiety should be free to interact with proteins in the membrane.

It was found that this derivative was not able to stimulate steroid production, suggesting that the drugs must be internalized in order to act.

Colchicine-like drugs shift the tubulin equilibrium toward the dimer and induce steroidogenesis without stimulating cyclic AMP production. Drugs such as taxol, that shift the equilibrium in favor of the polymer, were, however, found not only to counteract the steroidogenic effect of colchicine, but also the steroidogenesis induced by ACTH, cholera toxin or forskolin, drugs that increase cyclic AMP. It was shown that taxol did not prevent the rise in cyclic AMP. Vinblastine, another agent that promotes tubulin assembly, but to a different polymeric form, also inhibited steroid production without preventing the cyclic AMP increase. This suggests that tubulin's role in steroidogenesis may be separate from the process of kinase activation, the latter being a product of cyclic AMP activation. (Wolff, Sackett)

Studies on the possible role of Ca^{2+}/calmodulin in the regulation of microtubule assembly have been expanded. Calmodulin binding to microtubule-associated protein 2 (MAP2) occurs with a Kd of 7 μM and a stoichiometry of 1-1.5 moles per mole. By cross-linking ^{125}I-labeled calmodulin with MAP2 or with MAP2-microtubule adducts, and performing tryptic hydrolysis before or after cross-linking, results were obtained indicating that an approximately 37 kDa fragment of MAP2 is the site of calmodulin binding. This binding was shown previously to be calcium dependent. (Wolff, Lee)

Earlier studies showed that fluorescence occurs when colchicine binds to tubulin. This phenomenon has been reinvestigated in order to understand the mechanism of this effect. Although colchicine fluoresces in higher alcohols and shows a blue shift as a function of chain length, a further shift and an increase in fluorescence efficiency occurs in glycerol. This suggests that the viscosity rather than the dielectric constant of the solvent is the major factor and this was verified by measuring colchicine fluroescence in glycerol/dimethyl sulfoxide mixtures and other solvents. Furthermore, colchicine fluoresces when covalently coupled to proteins such as serum albumin, which do not promote fluorescence in the absence of covalent attachment. It is proposed that immobilization-enhanced fluorescence may be a more general phenomenon than heretofore believed. (Wolff, Bhattacharyya)

III. Polypeptide Hormones

A. Thyroid Stimulating Hormone (TSH)

The biosynthetic processing of TSH by carbohydrate incorporation and the regulation of this glycosylation has been the subject of continued investigation. [^3H]-Labeled carbohydrates were incubated with mouse thyrotropic tumor minces, and the products were analyzed in lipid extracts and in immunoprecipitated TSH using newly developed techniques of high performance liquid chromatography. Although lipid-linked oligosaccharides and total protein contained 2 to 3 terminal glucose residues, this was not the case with TSH subunits. The non-glucosylated core oligosaccharide ($Man_9GlcNAc_2$) was the predominant form after 10 min. pulse labeling but, under some incubation conditions (hypothyroid serum), $Glc_1Man_9GlcNAc_2$ was found.

The regulation of TSH biosynthesis was investigated since there has been little work on this aspect of TSH production. This was done with rat pituitary glands from normal or thyroidectomized rats, and incorporation of labeled glucosamine or alanine was examined. The effect of TRH was also studied. The results indicated that TSH synthesis was differentially regulated by thyroid hormone and TRH. With the development of hypothyroidism, synthesis of the β subunit apoprotein was stimulated and the α subunit became more highly glycosylated. TRH, on the other hand, had little or no effect on apoprotein synthesis but stimulated glycosylation of both subunits. Free α subunit was not greatly affected by either thyroid hormone or TRH. It is postulated that the alteration in glycosylation may have an important role in the biologic function of TRH. (Weintraub, Gesundheit, Burnside)

Further work has explored the question whether TSH obtained from the pituitary or the circulation in different physiological states might show different biological characteristics. The metabolic clearance of labeled TSH injected into rats varied with the source of the TSH and the thyroid status of the donor. Both serum-derived and pituitary-derived TSH showed an increased metabolic clearance rate, but in the former it was due to a lower disappearance rate while in the latter it was due to a smaller distribution volume. Control studies with normal and deglycosylated bovine TSH indicated that the carbohydrate content of the TSH from hypothyroid rats was probably the important factor determining the rate of clearance. From distribution studies it was seen that the kidney is the major organ of clearance. (Weintraub, Constant)

B. Gonadotropic Hormones

High performance liquid chromatography was also used to investigate the glycosylation of human chorionic gonadotropin (hCG) in cultured trophoblastic cells. Using endoglycosidase H to release oligosaccharide chains from the polypeptide backbone, it was found that the intracellular forms of hCG contained primarily the high mannose oligosaccharides. The rate-limiting step in processing appeared to be at the conversion of the Man_8 species to smaller forms. The processing of free α subunit and intact CG occurred at different rates, the former being somewhat slower. It was also found that intracellular CG is less bioactive than secreted forms, as expected for the incompletely glycosylated hormone.

In preliminary experiments on the regulation of CG synthesis, it was found to be increased 40 fold by a cyclic AMP analog, 8-Br cAMP. Free α-subunit synthesis was increased only 5 fold. In other newly initiated work, regulation of mRNA synthesis was studied in choriocarcinoma cells using full length cDNA probes to both hCG α and β subunits. Exposure to 8-Br cAMP increased mRNA synthesis 40 to 50 fold. (Rosen, Weintraub, Nagelberg, Burnside)

A rare form of gestational trophoblastic tumor was studied in a 32 year-old woman. Despite relatively low levels of hCG, the serum testosterone was 400 ng/dl, accounting for the patient's virilization. This was due to testosterone synthesis in the tumor as well as in the ovary. Another patient with poorly differentiated epidermoid carcinoma was studied in whom an unusual form of ectopic hCG-β was found. The molecular properties (high molecular weight, altered lectin binding, resistance to exoglycosidase, normal thermolysin "core" peptide molecular weight) suggested that the abnormal hCG might be hyperglycosylated. (Rosen, Nagelberg)

IV. Studies on Protein Structure and Interactions of Proteins with Cell Membranes

Studies have continued on the characterization of coated vesicles obtained from bovine brain. The principal protein is clathrin, which contains 3 heavy (180 kDa) and 3 light (30-35 kDa) chains. By examining its behavior in urea solutions, it was shown that there are at least two domains in the molecule with quite different stabilities. Enzymatic hydrolysis yielded a 110 kDa fragment that contains most or all of the organized structure of the 180 kDa chain.

The coated vesicles are involved in the endocytosis process of cells and studies have continued on the effects of agents known to interfere with endocytosis. Dissociation of clathrin from plasma membrane is inhibited by basic compounds, the order of effectiveness being spermine > spermidine > putrescine > propylamine. Very little difference, however, was observed in a series of dibasic amines of increasing chain length - i.e., $NH_2(CH_2)_{2-5}NH_2$. Anions of the Hofmesiter series were also studied. The results show that hydrophobic interactions contribute to stabilization of the clathrin coat structure in both coated vesicles and in "baskets" devoid of membrane. These anions also influenced the structure of the bilayer membranes in synthetic liposomes composed of dipalmitoyl- and dimyristyl-phospatidyl choline. (Edelhoch, Nandi, Prasad)

A possible role of coated vesicles in the thyroid follicular cell was investigated after incorporation of labeled methionine in bovine thyroid slices. Labeling of both thyroglobulin and clathrin was observed but no thyroglobulin was associated with coated vesicles. This argues against a role of these organelles in the movement of thyroglobulin through the cell. Thyroid coated vesicles were similar to but not identical with brain coated vesicles. (Edelhoch, Pierce)

V. Adenyl Cyclase of Bacterial Origin

Following the discovery that Bordetella pertussis adenyl cyclase can be activated by calmodulin in both a Ca^{2+}-dependent and a Ca^{2+}-independent manner, a method was devised for fractionating the enzyme on calmodulin affinity columns. The intracellular enzyme was extensively purified and its properties were shown to differ from those of the secreted enzyme. It has a smaller size, is stimulated by calmodulin and lipids, is unaffected by guanosine nucleotides and is inhibited by fluoride.

Administration of pertussis vaccine, consisting of whole, killed Bordetella pertussis organisms, causes hyperinsulinemia and enhanced secretion of insulin in response to a variety of secretagogues in rats and mice. Two distinct and separate effects of the bacteria on glucose and insulin levels in mice were found: a heat-stable component that causes a brief hyperinsulinemia and a second heat-labile component of the B. pertussis organism which induces a sustained (> 14 days), dose-dependent hyperinsulinemia and no associated hypoglycemia. The two effects are further distinguishable in that the early, endotoxin-induced hyperinsulinemia exhibits the normal suppressibility by exogenous epinephrine, whereas epinephrine markedly enhances the hyperinsulinemia occurring at 7 days. These two effects of B. pertussis appear to be mediated by different mechanisms and may be important in the well-recognized reactogenicity of pertussis vaccine in humans. (Wolff)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 45000-17 CEB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Thyroxine-Protein Interactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Robbins	Chief	CEB, NIADDK
Others:	H. Edelhoch	Chief, Prot. Struct. Sec.	CEB, NIADDK

COOPERATING UNITS *(if any)*
University of Rome, Rome, Italy (S. Grimaldi); University of Pisa, Pisa, Italy (L. Bartalena)

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Physiology Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: .7	PROFESSIONAL: .5	OTHER: .2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Further work has been done on the 27 kD protein obtained from human plasma as a contaminant of TBG preparations. The protein is immunochemically distinct from TBG, but binds T_4 with an association constant of 10^7. It, therefore, appears to participate in T_4 transport in blood. Studies on deglycosylation of TBG have shown that the oligosaccharide chains of TBG influence the structure of the apoprotein and also enhance its stability in acid and in guanidinium chloride solutions. T_4 binding, in addition to enhancing the stability of TBG, prevents the structural change that accompanies deglycosylation. These findings are among the first to define an interaction between the carbohydrate and amino acid moieties of glycoproteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 45004-13 CEB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure of Polypeptide and Protein Hormones

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. Edelhoch	Senior Scientist	CEB, NIADDK
Others:	R. E. Lippoldt	Sr. Health Services Officer	CEB, NIADDK
	K. Prasad	Visiting Fellow	CEB, NIADDK
	P. Nandi	Visiting Scientist	CEB, NIADDK
	L. R. Pierce	Medical Staff Fellow	CEB, NIADDK

COOPERATING UNITS *(if any)*

Endocrinology Department, Hospital of Foggia, Foggia, Italy (A. DiCerbo)

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Protein Structure Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.7	3.5	1.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The dissociation and denaturation properties of the principal coat protein clathrin have been studied in urea solution. Low concentrations of urea (< 3 M) dissociate the light chain, but no denaturation is observed. At higher urea concentrations, two distinct structural changes occur, one between 3 and 6 M and another above 7 M urea.

Basic compounds, e.g. biologic amines are found to stabilize the coat structure of clathrin in baskets as well as in coated vesicles. The degree of stabilization increases with the increase in the number of amino groups and does not depend upon the number of intervening methylene groups.

The contribution of the hydrophobic interaction toward the stability of the coat structure has been identified by the effect of lyotropic salts on the properties of the baskets and coated vesicles. The lyotropic salts affect the organized structure of the lipid vesicles (liposomes) similarly to that on proteins.

This study of [^{35}S]methionine incorporation into bovine thyroid slices suggests that coated vesicles do not have a major role in exocytosis of newly synthesized thyroglobulins from thyroid epithelial cells.

[^{35}S] incorporation into rat liver yields data which are compatible with the concept that the coat protein, clathrin, may be preferentially recycled during coated vesicle turnover as an indication of what may occur during receptor-mediated endocytosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 45009-17 CEB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies in Thyroid Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Robbins	Chief, Clin. Endocrin. Br.	CEB, NIADDK
Others:	E. Movius	Medical Staff Fellow	CEB, NIADDK
	L. R. Pierce	Medical Staff Fellow	CEB, NIADDK
	M. Phyillaier	Biologist	CEB, NIADDK
	J. Norton	Acting Head, Surg. Metab. Sec.	DCT, NCI

COOPERATING UNITS *(if any)*
Nuclear Medicine Department, CC, NIH (A. Keenan, R. A. Margolin, C. M. Clark, R. M. Kessler)

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Physiology Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.0	.4

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients in the thyroid carcinoma protocol undergoing follow-up whole body ^{131}I scanning were enrolled in a study of the effect of short-term hypothyroidism on glucose metabolism in the brain. Positron emission tomography with $[^{18}F]$-fluorodeoxyglucose was used to evaluate total glucose metabolism in 49 cerebral regions. Only one, the thalamus, showed metabolic rates significantly lower than that in the total cerebrum. This may be related to any inadequate relay of sensory input by the thalamic nuclei.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 45010-14 CEB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Ectopic and Eutopic Placental Proteins: In Vitro and In Vivo Studies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)*

PI:	S. W. Rosen	Medical Director	CEB, NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Physiology Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Terminated - Refer to Project No. Z01 AM 45030-04 CEB for continuation of this project.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 45013-12 CEB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biosynthesis and Glycosylation of Thyrotropin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. D. Weintraub	Med. Dir.	CEB, NIADDK
Others:	B. S. Stannard	Biologist	CEB, NIADDK
	T. Taylor	Guest Researcher	CEB, NIADDK
	N. Gesundheit	Medical Staff Fellow	CEB, NIADDK
	J. Burnside	Staff Fellow	CEB, NIADDK

COOPERATING UNITS *(if any)*
Dept. of Medicine, Michael Reese Hospital, Chicago, Illinois (J. Magner);
Faculty of Medicine, University of Marseille, Marseille, France (C. Ronin)

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Physiology Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.9	2.9	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Thyrotropin glycosylation has been characterized during pulse-chase labeling using newly developed methods of high performance liquid chromatography. Inhibitors of glucosidase block TSH carbohydrate processing at different steps yielding abnormally glycosylated TSH forms whose functional characteristics are being investigated. Thyroid hormone and thyrotropin-releasing hormone differentially regulate thyrotropin apoprotein and carbohydrate biosynthesis. Apoprotein biosynthesis is primarily regulated in the free β subunit while carbohydrate biosynthesis is primarily modulated in the combined α subunit.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 45014-13 CEB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Membranes and Secretion (formerly Thyroid Membranes and Secretion)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)*

P. I.:	J. Wolff	Associate Chief	CEB, NIADDK
Others:	D. Sackett	Staff Fellow	CEB, NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Clinical Endocrinology Branch

SECTION

Endocrine Biochemistry Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.4	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Evidence has been accumulated that indicates that cAMP is not involved directly in the steroidogensis effect of antimicrotubule agents and the protein kinase step in adrenal cell activation appears to be circumvented.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 45016-14 CEB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Thyroid Hormone Secretion and the Function of Microtubules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Wolff	Associate Chief	CEB, NIADDK
Others:	Y. C. Lee	Staff Fellow	CEB, NIADDK
	B. Bhattacharyya	Visiting Scientist	CEB, NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Biochemistry Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.3	.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The calcium-dependent calmodulin interaction of microtubule-associated protein 2 MAP2 has been studied by cross-linking of chymotryptic fragments of MAP2. Several approaches show that the calmodulin attachment site is on the short arm of MAP2 that binds to tubulin and not on the long side arm that interacts with cellular organelles.

We have also reinvestigated the basis for the fluorescence that occurs on binding of colchicine to tubulin. This is not primarily a function of the hydrophobic environment provided by the binding site but is a function of the immobilization of the drug in the site and can be mimicked by high viscosity, protein-free, media or by non-specific covalent coupling to proteins that do not normally bind colchicine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 45018-09 CEB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Adenylate Cyclase and Other Extracellular Products of B. Pertussis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Wolff	Associate Chief	CEB, NIADDK
Others:	B. Neumeyer	Bio Lab Tech	CEB, NIADDK

COOPERATING UNITS *(if any)*
Department of Medicine, University of Virginia, Charlottesville, Virginia (E. L. Hewlett); Laboratory of Cellular and Developmental Biology, NIADDK, NIH (C. Londos)

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Biochemistry Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.3	.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A new form of Bordetella pertussis adenylate cyclase has been purified to homogeneity and shown to differ markedly from the previously isolated cyclase from the culture medium in molecular weight, isoelectric point and stimulability with calmodulin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 45020-08 CEB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Synthesis of Thyroxine Transport Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Robbins	Chief, Clin. Endocrin. Br.	CEB, NIADDK

COOPERATING UNITS *(if any)*
University of Pisa, Pisa, Italy (L. Bartalena); University of Rome, Rome, Italy (S. Grimaldi)

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Physiology Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.4	.2	.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The 27 kD protein previously observed during the study of thyroxine-binding globulin (TBG) synthesis by human hepatocarcinoma cells has been shown to be distinct immunochemically from TBG. Its biosynthesis is directed by messenger RNA distinct from TBG mRNA. The 27 kDa protein is able to bind T_4 and thus represents a previously unrecognized thyroid hormone transport protein of human plasma.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 45021-08 CEB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Clinical Pathophysiology of Pituitary Gonadotropins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)*

PI:	S. W. Rosen	Medical Director	CEB, NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Physiology Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Terminated

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 45023-08 CEB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Thyroid Hormone and Thyrotropin Receptor Binding and Action

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. D. Weintraub	Medical Director	CEB, NIADDK
Others:	M. Menezes-Ferreira	Visiting Fellow	CEB, NIADDK
	R. Constant	Guest Researcher	CEB, NIADDK
	P. Petrick	Medical Staff Fellow	CEB, NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Clinical Endocrinology Branch

SECTION

Endocrine Physiology Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.7	3.3	.4

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A large number of patients with both neoplastic and non-neoplastic inappropriate TSH production have been identified using novel diagnostic methods. The molecular basis for these disorders is under investigation and new therapeutic approaches are being explored. Inappropriate TSH action has been discovered in patients with central hypothyroidism. The biologically inactive TSH in this disease results from impaired binding to its receptor and the bioactivity can be restored by chronic treatment with thyrotropin-releasing hormone. In rats, various hormones regulate TSH bioactivity, metabolic clearance and targeting; studies with chemically deglycosylated TSH suggest that these effects are mediated by changes in carbohydrate structure. Functional primary cultures of human and porcine thyroid follicles are being developed to define the mechanisms of action of various thyrotropic hormones and immunoglobulins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 45025-06 CEB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Effects of Triiodothyronine on Liver Nucleoproteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	V. M. Nikodem	Senior Staff Fellow	CEB, NIADDK
Others:	H. Cahnmann	Scientist Emeritus	CEB, NIADDK
	B. Dozin	Visiting Fellow	CEB, NIADDK
	M. Magnuson	Medical Staff Fellow	CEB, NIADDK

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Protein Structure Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Terminated

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 45028-06 CEB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Thyroid Hormone-Cell Interactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Robbins	Chief, Clin. Endocrin. Br.	CEB, NIADDK
Others:	E. Movius	Medical Staff Fellow	CEB, NIADDK
	A. Pontecorvi	Visiting Fellow	CEB, NIADDK
	M. Phyillaier	Biologist	CEB, NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Biochemistry Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.8	1.8	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mechanism of thyroid hormone entry into cells is under study, utilizing intact rat skeletal muscle, monolayer cell cultures of rat myocytes and human hepatocarcinoma cells, and an artificial membrane composed of dipalmitoyl phosphatidyl choline (DPPC). Kinetics of thyroxine (T_4) and triiodothyronine (T_3) binding to DPPC vesicles showed more rapid entry of hormone when the lipid was in the liquid-crystal phase compared to the gel phase ($T_{1/2}$ 10 to 13 sec compared to 36 to 55 sec). The greater lipophilicity of T_4 compared to T_3 results in faster uptake of T_4 when the lipid is in the gel phase. In the intact skeletal muscle as well as in cultured hepatic and skeletal muscle cells, about one-third of the hormone uptake is by a saturable, energy-dependent process that presumably accounts for at least part of the entry of hormone into cells _in vivo_.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 45029-04 CEB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

POEMS Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)*

PI:	S. W. Rosen	Medical Director	CEB NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Clinical Endocrinology Branch

SECTION

Endocrine Physiology Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Terminated

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 45030-04 CEB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biosynthesis and Glycosylation of Human Chorionic Gonadotropin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. D. Weintraub	Medical Director	CEB, NIADDK
Other:	S. W. Rosen	Medical Director	CEB, NIADDK
	I. Calvert	Chemist	CEB, NIADDK
	S. Nagelberg	Medical Staff Fellow	CEB, NIADDK
	J. Burnside	Staff Fellow	CEB, NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Clinical Endocrinology Branch

SECTION

Endocrine Physiology Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
3.5	2.3	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Human chorionic gonadotropin glycosylation is being characterized in cultured human trophoblastic cell lines using newly developed methods of high performance liquid chromatography. Regulation of chorionic gonadotropin synthesis is being studied at the levels of transcription, messenger RNA stability, translation and post-translational processing. Human tumors may secrete aberrant forms of chorionic gonadotropin, which may have important diagnostic and functional implications.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 45031-04 CEB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Photolysis of Thyroxine and Thyroxine Analogs

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	H. J. Cahnmann	Scientist Emeritus	CEB, NIADDK
Others:	V. M. Nikodem	Senior Staff Fellow	CEB, NIADDK
	B. Dozin	Visiting Fellow	CEB, NIADDK

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Endocrine Biochemistry Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	.9	.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Irradiation of rat liver nuclear extract after preincubation with [^{125}I]thyroxine with light of high intensity (near U.V. and visible regions) for ~ 1 min resulted in covalent labeling of the thyroid hormone receptor(s). Covalent labeling efficiency was the same as that achieved after irradiation for 30 min, when the previously described photolabeling procedure was used.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 45033-01 CEB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mapping of Triiodothyronine Responsive Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	V. M. Nikodem	Senior Staff Fellow	CEB, NIADDK
Others:	M. A. Magnuson	Medical Staff Fellow	CEB, NIADDK
	M. Tecce	Visiting Fellow	CEB, NIADDK

COOPERATING UNITS (if any)

Genetics and Biochemistry Branch, NIADDK, NIH (D. Camerini-Otero)

LAB/BRANCH

Clinical Endocrinology Branch

SECTION

Protein Structure Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.5	1.3	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

1. Using cDNA clones for rat malic enzyme and two other unidentified rat liver messenger ribonucleic acids which are highly responsive to the thyroid hormones, we have probed a genomic library in bacteriophage Charon 4A. Genomic clones for these cDNAs were obtained.

2. A triiodothyronine stimulated rat liver cDNA library and a bovine liver cDNA library were constructed in the expression vector bacteriophage λgt 11.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 45034-01 CEB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Specific Rat Liver mRNAs by Thyroid Hormone

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	V. Nikodem	Senior Staff Fellow	CEB, NIADDK
Others:	M. Magnuson	Medical Staff Fellow	CEB, NIADDK
	B. Dozin	Visiting Fellow	CEB, NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Clinical Endocrinology Branch

SECTION
Protein Structure Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.6	1.4	.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1. A plasmid cDNA library was constructed using poly(A^+)RNA isolated from the livers of rats treated with triiodothyronine and fed a high carbohydrate diet. The library was screened by differential colony hybridization and dot blot hybridization using ^{32}P-cDNA probes made from hypothyroid and hyperthyroid rat liver poly(A^+)RNA. cDNAs for 8 different T_3 responsive mRNAs were obtained. Then they were used to determine: a) relative mRNA level in different thyroid states and response to a high carbohydrate diet; b) kinetic response to receptor saturating doses of triiodothyronine in hypothyroid animals. Several of these cDNA clones may serve as models for the study of the mechanism of action of T_3 on the regulation of gene expression.

2. We have demonstrated by hybridization assays with malic enzyme cDNA that: a) liver malic enzyme synthesis is directed by two distinct and functional cytoplasmic mRNAs; b) malic enzyme activity is modulated at the pretranslational level by thyroid hormones and carbohydrate diet, presumably through regulation of the rate of malic enzyme gene transcription; c) the level of expression of malic enzyme gene as well as its regulation by T_3 are tissue specific and the hormonal stimulation affects both malic enzyme mRNAs to a similar degree in the T_3 responsive tissues (liver, heart and kidney).

3. Preliminary results show that T_3 regulates the rate of transcription of specific genes to some extent.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 45035-01 CEB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Photoaffinity Labeling of Thyroid Hormone-Specific Binding Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)*

PI:	V. M. Nikodem	Senior Staff Fellow	CEB, NIADDK
Others:	H. J. Cahnmann	Scientist Emeritus	CEB, NIADDK
	B. Dozin	Visiting Fellow	CEB, NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Clinical Endocrinology Branch

SECTION

Protein Structure Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 1.1	PROFESSIONAL: .9	OTHER: .2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Photoaffinity labeling using underivatized $[^{125}I]T_4$ or $[^{125}I]T_3$ is highly specific. Therefore, peaks of radioactivity obtained after sodium-dodecylsulfate gel electrophoresis indicate that labeled proteins have thyroid hormone binding sites.

Alumni of the Diabetes Branch continue to prosper. Dr. Pierre De Meyts received the Assubel Scientific Prize awarded by the Queen of Belgium.

A fellowship was received from the Pharmacology Research Associate Program of the National Institute of General Medical Sciences at the NIH.

Diabetes Branch members have received recognition by special lectureships. These include the James Grant Thompson Memorial Lectureship of the Mississippi State Medical Association (P.G.) and the Karl Beyer Lectureship of the W. S. Middleton Memorial V. A. Hospital in Madison, Wisconsin (J.R.).

INSULIN RECEPTORS IN RELATED HORMONES

Phosphorylation of the Insulin Receptor:

It is now clear that the alpha subunit of the insulin receptor is the major binding subunit of the receptor and the beta subunit is a protein kinase, capable of autophosphorylating itself and a number of exogenous substrates. Many details of this phosphorylation reaction in both blood cells and liver have been under continued study. It is known that the phosphorylation reaction can be regulated by divalent cations, ATP, and most importantly, insulin itself. Recent studies have indicated that the biosynthetic precursor of the receptor, i.e. the single chain molecule is capable of undergoing phosphorylation. Further, the IGF-I receptor, which is structurally similar to the insulin receptor, also undergoes a ligand induced autophosphorylation of the beta subunit of this receptor. Insulin receptors are present in the central nervous system, though their function is as yet unclear. These receptors, like peripheral hormone receptors, also undergo a similar autophosphorylation event. Since autoantibodies to the insulin receptor simulate many or all of the biological effects of insulin, it is impossible to ask whether phosphorylation is required for the action of these antibodies. We have observed at least two antibodies capable of stimulating insulin biological effects but do not stimulate autophosphorylation of the insulin receptor. The explanation for this is as yet unclear but it raises the question of whether or not phosphorylation is an obligatory event in insulin action. It is now possible to study phosphorylation in experimental situations of hormone resistance. Two situations have thus far been studied, dexamethasone-treated rats and 72 hours of fasting in rats. Both situations result in insulin resistance but phosphorylation of the receptor does not appear altered under these experimental circumstances.

Insulin Binding in Circulating Cells

Clinical insulin resistance has continued to be studied with specific regard to the insulin receptor. Both peripheral blood monocytes and erythrocytes have been useful cells to study this reaction. In previous studies the focus has been on the alpha subunit (the binding subunit) of the receptor. More recent studies have focused on the beta subunit (the protein kinase subunit) of the receptor. We have demonstrated a marked discordance in binding and protein kinase activity in one patient with the Type A form of insulin resistance. These data raise the question of whether or not a coupling mechanism has been lost in this patient and therefore is related to the patient's insulin resistance. Other Type A patients, either with normal binding or low binding, demonstrate a general concordance of binding and phosphorylation in their peripheral blood mononuclear cells.

We have shown that the tumor-promoting phorbol esters which are known to be serine kinases in other cell types, can stimulate specific tyrosine kinase activity in peripheral blood monocytes and in the U-937 monocyte cell line. The site of this phosphorylation appears to be the alpha subunit of the insulin receptor cell. It is known also that these tumor-promoters will decrease the affinity of insulin for its receptor. These studies raise the possibility of a mechanism that links the tumor-promoting phorbol ester to the polypeptide growth factors, i.e. both participate in a tyrosine specific phosphorylation event.

Other studies have attempted to define the mechanism whereby sulfonylureas may alter insulin sensitivity. We find that these drugs do not effect the insulin receptor directly, but presumably produce any benefit through some indirect effect. We are continuing to carry out a controlled clinical trial of the sulfonylurea as an inducer of a new steady-state to see whether this can be maintained for a prolonged period of time on placebo therapy. This might lead to the use of these drugs on an interim basis with appropriate metabolic control.

Cellular Hormone-like Peptides and Embryo Receptors

Insulin-related peptides have been isolated from the unicellular eukaroytic organism Tetrahymena and bacteria including E. coli. In addition, several other polypeptide hormones such as ACTH, somatostatin, and the endorphin-type peptides as well as relaxin, have been isolated from these sources. Additional studies are in progress to characterize these peptides. For instance, it has now been demonstrated that small amounts of insulin-related activity can be extracted from plasmid-free bacteria. Further work along this line has demonstrated insulin-related material in high plants. At this point the insulin-related material appears to be typical of mammalian types of insulin by HPLC characterization, immunoreactivity, and now biological activity studies.

Studies that had initially demonstrated the presence of insulin receptors in rat brain have been extended to chick embryo brains. Insulin receptors can be detected in heads and bodies of embryos by 4 days of incubation and that binding continues in a linear fashion as a function of gestation. By contrast, growth factor binding can be detected as early as day 2 and reaches a plateau by day 12 with a decrease in binding by day 18 of development.

Further studies have been carried out in Chinese hamster and rodent model systems. The general conclusion from these studies is that tissue insulin appears to be partially independent of plasma insulin, that is, the concentrations do not mirror the concentrations of plasma insulin under a variety of circumstances where plasma insulin is either raised or lowered. This is with the exception of the kidney which appears to mirror well the changes in plasma insulin activity. These studies have raised the question of whether extra-pancreatic sources are capable of synthesizing insulin for some purpose which is presumably distinct from metabolic control.

Morphologic Studies of Ligand Binding to Cells

These investigations are a continuing collaboration with the University of Geneva. Recent studies demonstrate that receptor-mediated endocytosis is a relatively non-specific phenomenon with respect to the structures involved in the endocytosis process. For instance, cationic ferritin binds to coated pits at the surface of cells and is endocytosed into exactly the same internal compartments as is seen for ^{125}I-insulin. Thus, the receptor and some event controlling recycling represents sites of specificity. Further, it has been shown that in circulating monocytes isolated from patients and in the U-937 monocyte-like cell line that insulin is internalized in an analogous fashion to that seen in the hepatocyte. Thus, it appears that receptor regulation in peripheral blood cells is analogous to that seen in hepatocytes and that these cells should mirror changes in ambient insulin concentration. While these cells have been used extensively over the years as models of insulin action at the receptor level, it has not been previously clear how insulin serves to regulate its receptor on these presumed non-target tissues. Further studies have demonstrated that different cell lines, the U-937 monocyte which internalizes insulin avidly and the IM-9 lymphocyte which internalizes insulin poorly, have different rates of recycling of the insulin receptor. The U-937 cells have a marked augmented down regulation when the ionophore monensin is added with insulin under down regulating conditions. This suggests that a block in receptor recycling is a major additive effect in promoting down regulation. Monensin, on the other hand, in the IM-9 cell, had no additive effect, consistent with the very low internalization rate seen in this cell. Still other studies have defined an endosomal compartment in the hepatocyte and suggest that the ligand is directly transferred from the cell membrane into this endosomal compartment and subsequently through a compartment which is lysosome-like, that is, multivesicular bodies and more typical lysosomal structures.

Insulin Receptors in Syndromes of Extreme Insulin Resistance

Using the Epstein-Barr virus-transformed lymphocyte, it has previously been shown that patients with a variety of insulin resistant syndromes manifest either normal or low insulin binding. Since the receptor concentration on the surface of the cells is a function of receptor degradation and receptor synthesis both of these parameters have been studied. Cells whose insulin receptors were labeled with ^{125}I at the cell surface and returned to the incubation media, can degrade the receptor with a half-life of approximately 6.5 hours. By contrast, in cells from insulin resistant patients, whether binding was normal or low, insulin receptor degradation was moderately elevated to the extent of $\sim 40\%$. Thus this is a new abnormality not previously demonstrated in the insulin resistant patients, that is, acccelerated receptor degradation. This accelerated receptor degradation, however, cannot account for the extremely low binding found in some of these patients.

Biosynthetic studies have been carried out and abnormalities of at least three different types have been demonstrated. The most common abnormality appears to be a decrease in all components of the receptor, i.e. the precursor as well as the mature subunits. A second defect involves an abnormal precursor molecule. A third defect involves a receptor which appears to be synthesized in a roughly normal fashion but is not inserted into the plasma membrane. It has further been shown that the EB-virus-transformed lymphocytes undergo down regulation *in vitro*. This is in contrast to behavior of their monocytes *in vivo* where very high insulin concentrations in the blood are not accompanied by appropriate degrees of down regulation.

Autoantibodies to the Insulin Receptor

Insulin receptor autoantibodies have been partially purified and, as previously mentioned, shown to have insulin-like activity *in vitro*. It was important to verify the *in vivo* activity of the antibodies by developing a test system to study their action. A rat was used as a model system. It could be shown that acute injection of a partially purified anti-receptor antibody into a fasted rat produced slow but progressive hypoglycemia. By contrast, when multiple injections were given to fed animals, the initial hypoglycemic response changed to hyperglycemia with a sustained diabetic syndrome. It appears that the clinical features of this disease can be explained by different activities of the same antibody at the cellular level. Further, *in vitro* studies have demonstrated that the autoantibodies are capable of down regulating the insulin receptor and this may be, in part, the mechanism by which the insulin effect is generated by these antibodies.

Studies with monoclonal antibodies against insulin to probe the domains of the insulin receptor have been carried out. These data suggest that most of the epitopes of the insulin molecule becomes sterically unavailable for the antibody binding. It seems likely that the hormone binding site of the receptor may be very large thereby allowing for sequestration of the majority of the insulin molecule with relatively little of the hormone remaining exposed.

Growth Hormone and Acromegaly

Using standard cross-linking techniques, the growth hormone receptor of the IM-9 lymphocyte has been identified as an approximate 140K protein. Since the insulin receptor is capable of autophosphorylating itself, it was of interest to investigate whether this was also true for the growth hormone receptor. Thus, a variety of conditions known to be optimal for phosphorylating the insulin receptor were used in an attempt to demonstrate growth hormone phosphorylation of the 140K protein. None of the conditions used produced phosphorylation. Since phosphorylation of receptors is also achieved by several growth factors that bind to cells, and since insulin as well as these insulin-like growth factors are internalized by cells, we questioned whether or not phosphorylation is important for receptor mediated endocytosis. The data from the studies using growth hormone which is avidly internalized by IM-9 lymphocytes but does not phosphorylate its receptor suggests that phosphorylation is not an essential event in receptor mediated endocytosis.

Additional studies have been carried out on the heterogeneity of growth hormone components in plasma. We find that the 22,000 major growth hormone plasma component disappears at a faster rate than the higher molecular weight components (demonstrated by gel filtration). The higher molecular weight components are known to be biologically less active. Therefore, growth hormone from plasma is consistent with the receptor-mediated process.

Biosynthetic Labeling of the Insulin Receptor

Using both labeled sugars and amino acids, it has been demonstrated that the initial form of the insulin receptor is a 190K protein. This protein contains glycoproteins in the high mannose form and is presumably the result of co-translational core glycosylation of a newly formed polypeptide. The 190K precursor reaches a peak at approximately 1 to 1.5 hours and then its concentration is reduced. Starting at about 2 hours, the typical 135K and 95K subunits of the insulin receptor are seen. Thus, several events are important in the biosynthesis of the insulin receptor. These include initial formation of a protein backbone and glycosylation of a protein resulting in a precursor molecule. The molecule must then undergo proteolytic cleavage and further processing to a complex carbohydrate form which is the natural form of the receptor as it exists in the cell membrane. These studies have provided an important background for studying receptor defects and have provided a model system for studying the very low binding cell lines of patients with insulin receptor diseases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 47001-03 DB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Phosphorylation of the Insulin Receptor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	R. W. Rees-Jones	Medical Staff Fellow	DB, NIADDK
Others:	G. Grunberger	Clinical Associate	DB, NIADDK
	Y. Zick	Research Associate	Israel
	M. Quarum	Bio. Lab. Tech.	DB, NIADDK
	P. Gorden	Chief	DB, NIADDK
	J. Roth	Director, DIR	NIADDK

COOPERATING UNITS *(if any)*
Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel.

LAB/BRANCH
Diabetes Branch

SECTION
Receptors and Hormone Action Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Insulin enhances phosphorylation of both alpha and beta subunits of its own receptor by stimulation of tyrosine-specific protein kinase which is tightly associated with the receptor itself. The biochemical characterization of this enzyme is being studied under several conditions in order to determine its potential physiological significance. Although the enzyme is tightly associated with the receptor, the phosphorylating machinery can be distinguished from the insulin binding site itself. Studies in tissue (liver and brain) from rats under two conditions of corticosteroid excess and fasting in which there is a defect in insulin action distal to insulin binding have been carried out. Post-binding defect in insulin action can not be explained by impairment of intrinsic activity of insulin receptor-associated protein kinase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 47005-12 DB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of Insulin Binding in Circulating Cells in Man

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	G. Grunberger	Clinical Associate	DB, NIADDK
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	S. I. Taylor	Medical Officer	DB, NIADDK
	J. L. Young	Bio. Lab. Tech	DB, NIADDK
	P. Gorden	Chief	DB, NIADDK

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Diabetes Branch

SECTION
Clinical and Cellular Biology Section; Cellular and Molecular Physiology Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Numerous studies from this laboratory have investigated the interaction of insulin with circulating mononuclear cells. We have attempted to characterize the insulin receptor of the human erythrocyte. Mononuclear cells and erythrocytes both have insulin receptors whose functions are altered by conditions of health and disease. Comparative studies of these two different cell types show that in some cases their insulin receptor status may be similar, but that the actual regulation of their receptor concentration may differ. The circulating monocyte is being used to study the function of alpha and beta subunit of the insulin receptor. One patient with insulin resistance shows discordance between insulin binding and insulin stimulated tyrosine-kinase activity. Six patients with Type A syndrome of insulin resistance shows concordance between insulin binding and insulin stimulated phosphorylation. The physiological role of receptor-associated tyrosine kinase activity remains unclear. The effects of sulfonylurea drugs on erythrocytes and monocytes in Type II diabetes are also under study.

Further, it has been shown that the tumor promoting phorbol esters can stimulate tyrosine phosphorylation in circulating monocytes and in the U-937 monocyte cell line. This may represent an important link of these tumor promoters and polypeptide growth factors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 47007-09 DB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Antibodies to Receptors: Detection in Disease States and Use as Probes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P. I.	S.I.Taylor	Med.Off.	DB/NIADDK			
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	N.Perrotti	V.A.	DB/NIADDK	J. Schroer	V.A.	LIG/NIAID
	J.A.Hedo	V.A.	DB/NIADDK	I.Pastan	Chief	LMB/NCI
	Y.Zick	R.A.	Weismann Inst.	N.Riechert	S.I.	LMB/NCI
	R.Rees-Jones	M.S.F.	DB/NIADDK	U.Rapp	AcChief	LVC/FCRF
	J.Roth	Dir.	IRP/NIADDK	T.Bender	S.F.	NCI-Navy/NHB
	P.Gorden	Chief	DB/NIADDK	R.F.Dons	C.A.	DB/NIADDK
				R.Havlik	CoStep	DB/NIADDK

COOPERATING UNITS *(if any)*

Weismann Institute
NIAID:LIG
NCI:LMB
FCRF:LVC
NHB:NCI-Navy

LAB/BRANCH

Diabetes Branch

SECTION

Clinical and Cellular Biology Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.2	2.7	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Autoantibodies to the insulin receptor occur in patients with insulin resistance as well as patients with hypoglycemia. Both in vitro and in vivo studies on the effects of anti-receptor antibodies to mimic or antagonize insulin action have been studied. A recent discovery shows that anti-receptor antibodies can mimic insulin's effect to down-regulate receptors by accelerating their degradation. This effect of anti-receptor antibodies may play an important role in the desensitization of the target cell to the effects of insulin and to the insulinomimetic effects of anti-receptor antibody.

When intact rat adipocytes are incubated with anti-receptor antibodies, the antibodies mimic the metabolic effect of insulin without stimulating phosphorylation of the receptor. Four antisera mimic insulin action in vitro, suggesting that the ability to stimulate the receptor-associated protein kinase may not play a role in mediating the insulinomimetic action of anti-receptor antibodies.

Anti-insulin antibodies have been used as a probe for the interaction between insulin and its receptor. Polyclonal anti-insulin antibodies can immunoprecipitate the cross-linked ^{125}I-insulin-receptor complex. Sixteen monoclonal antibodies failed to immunoprecipitate receptor-bound ^{125}I-insulin. One monoclonal antibody immunoprecipitated the cross-linked ^{125}I-insulin-receptor complex, albeit poorly. These data suggest that the majority of the epitopes on the insulin molecule become sterically unavailable for antibody binding.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 47014-15 DB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Acromegaly and Growth Hormone

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	P. Gorden	Chief	DB, NIADDK
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	C. M. Hendricks	Bio. Lab. Tech.	DB, NIADDK
	N. Hizuka	Professor	Japan
	G. Grunberger	Clinical Associate	DB, NIADDK
	A. McElduff	Visiting Associate	DB, NIADDK

COOPERATING UNITS *(if any)*

Department of Medicine, Tokyo Women's Medical College, Tokyo, Japan

LAB/BRANCH

Diabetes Branch

SECTION

Clinical and Cellular Biology Section; Receptors and Hormone Action Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Acromegalic patients have continued to be followed with respect to pituitary irradiation. Further, we are evaluating the effects of transphenoidal hypophysectomy in these patients and comparing them to the pituitary-irradiated patients.

When bromocryptine was used as an adjunct or as primary therapy for acromegaly, it was found that approximately one-third of the patients had some reduction in growth hormone concentration but there was no alteration in the form of the growth hormone components in circulating blood.

Recently, human growth hormone has been cross-linked to its specific cellular receptor on IM-9 cultured lymphocytes. Under reducing conditions the predominant band is an approximate 140K protein. Under non-reducing conditions, however, a molecular weight component greater than 200K is observed. Whether this represents an oligomer of the reduced peptide is uncertain at this point. Further attempts were made to see if the growth hormone receptor was a protein kinase or a substrate for this kinase activity, it does not. Thus, the growth hormone receptor is not analogous to the insulin receptor.

The heterogeneity of circulating growth hormone in plasma has been studied. Pituitary growth hormone was injected in normal volunteers and the individual growth hormone components isolated by gel filtration. It was found that the half-time of the "little" (22,000 Da) growth hormone component was faster than the "big" and the "pre-big" growth hormone components, respectively. This is compatible with a receptor-mediated type of removal of these components since previous studies have shown that the high molecular weight forms have lower radioreceptor activity than the 22,000 growth hormone preparation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 47018-07 DB

PERIOD COVERED
October 1, 1983 to September, 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cellular Hormone-Like Peptides and Embryo Receptors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Jesse Roth, M.D.		
Other Personnel:	D. LeRoith,	Visiting Scientist,	DB,NIADDK
	M. A. Lesniak,	Chemist,	DB,NIADDK
	J. Shiloach,	Visiting Scientist,	A, LCDB
	C. Rubinovitz,	Visiting Fellow,	A, LCDB
	E. Collier,	Medical Staff Fellow,	DB,NIADDK
	N. Weaver,	Biomedical Technician	DB,NIADDK
	A. Watkinson,	Visiting Fellow,	DB,NIADDK

COOPERATING UNITS *(if any)*

Laboratory of Cellular and Developmental Biology, NIADDK

LAB/BRANCH
Diabetes Branch

SECTION
Receptors and Hormone Action Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 5.0	PROFESSIONAL: 4.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Hormone-related (insulin, somatostatin; beta endorphins, relaxin, etc.) materials have been found in extracts of Tetrahymena grown in defined media; insulin-related material also are found in extracts of E.coli. These studies of hormone-like materials have been extended to the plant kingdom. Insulin-related materials immunoactivity have been found in plants, extracts of spinach, lemna and rye. These insulin-related materials have been characterized by ion exchange, gel, and high performance liquid chromatography and in a bioassay using rat adipocytes. These findings suggest that hormone-like materials are evolutionarily very old.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 47019-07 DB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Morphologic Studies of Ligand Binding to Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	P. Gorden	Chief	DB, NIADDK
Others:	G. Grunberger	Clinical Associate	DB, NIADDK
	J.-L. Carpentier	Research Associate	Switzerland
	A. Robert	Research Associate	Switzerland
	J.-Y. Fan	Research Fellow	Switzerland
	L. Orci	Professor of Med.	Switzerland

COOPERATING UNITS *(if any)*
Institute of Histology and Embryology, University of Geneva, Geneva, Switzerland

LAB/BRANCH
Diabetes Branch

SECTION
Clinical and Cellular Biology Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Quantitative EM autoradiography has been used as a visual probe. In an attempt to use a higher resolution probe in hormone binding studies cationic ferritin has been employed. Cationic ferritin initially binds to the villous surface of the cell, is subsequently localized in coated pits and then internalized. The material is internalized first into coated vesicles and then into a series of membrane bounded vesicles of increasing size. This is analogous to the pattern that has been observed with iodide labeled insulin in 3T3-L1 adipocytes. The process of receptor-mediated endocytosis appears to be relatively non-specific with respect to the various structures involved.

Previously an autoantibody to the insulin receptor, when labeled with iodine and cytoabsorbed for purification, binds to cell surfaces and is internalized in an analogous fashion to insulin. Recent studies show that the univalent Fab fragment is processed in an identical fashion to both insulin and to the divalent receptor antibody.

The U-937 monocyte-like cultured cell, a model for circulating monocytes, binds and internalizes insulin in an analogous fashion to target cells such as liver cells. The U-937 monocyte internalizes insulin at a high rate and the addition of monensin to insulin markedly augments down regulation of receptor. By contrast, the IM-9 lymphocyte which internalizes insulin poorly, shows essentially no additional effect when monensin is added. The drug colchicine which inhibits endosomal transfer to lysosomes in hepatocytes has been studied. There is no localization to an additional compartment such as the Golgi with respect to processing of an internalized ligand. The internal route appears to go from the endosome of one or more types to the lysosome of one or more types; the recycling process would appear to occur from either of the two membrane-bounded structures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 AM 47021-06 DB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Receptors and Hormone Action in Cultured Human Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	J. M. Podskalny	Biologist	DB, NIADDK
Others:	A. McElduff	Visiting Associate	DB, NIADDK
	D. G. Rouiller	Visiting Associate	DB, NIADDK
	S. Takeda	Visiting Associate	DB, NIADDK
	N. Sharon	Visiting Scientist	DB, NIADDK
	R. E. Silverman	Medical Staff Fellow	DB, NIADDK
	G. Grunberger	Clinical Associate	DB, NIADDK
	P. Gorden	Chief	DB, NIADDK

COOPERATING UNITS (if any)
Institute of Histology and Embryology, University of Geneva, Geneva, Switzerland

LAB/BRANCH
Diabetes Branch

SECTION
Cellular and Molecular Physiology Section; Clinical and Cellular Biology Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.7	4.7	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Two types of cultured cells, human hepatoma cells (Hep G2) and cultured human fibroblasts have been previously well characterized as regards their insulin receptors. The Hep G2 cells have now been evaluated in several bioassays for insulin responsiveness: glucose uptake, glycogen synthase activation, thymidine incorporation into DNA, uridine incorporation into RNA and carbon dioxide production from acetate. Insulin receptors from both Hep G2 cells and human fibroblasts have been solubilized and receptor and/or artificial substrate phosphorylation measured. The status of insulin stimulated phosphorylation using fibroblasts from several insulin resistant patients is under investigation. The insulin receptor in Chinese hamster ovary (CHO) cells have been identified and partially characterized. Two mutant CHO cell lines have been used to examine the role of glycosylation in receptor function and in receptor processing. Receptor glycosylation may be involved in receptor binding and may influence receptor affinity. The insulin receptor biosynthesis and insertion into the plasma membrane appears to occur normally even in mutant cell lines with major defects in receptor glycosylation. Lectins have been used as probes to determine glycosylation status of the insulin receptor in CHO cells and their mutants.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 47022-05 DB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Insulin Receptors in Syndromes of Extreme Insulin Resistance

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	S.I.Taylor	Med.Off.	DB/NIADDK
Others:	G.Grunberger	C.A.	DB/NIADDK
	Y.Zick	R.A.	Weizmann Inst.
	J.A.Hedo	V.A.	DB/NIADDK
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	B. Samuels	C.	DB/NIADDK
	N.Perrotti	V.A.	DB/NIADDK
	R.Comi	M.S.F.	DB/NIADDK
	J.Young	BLT	DB/NIADDK
	C.M.Hendericks	BLT	DB/NIADDK
	J.Roth	Dir.	DIR/NIADDK
	P.Gorden	Chief	DB/NIADDK
	L.D.Wagman	Chief	SB/NCI

COOPERATING UNITS *(if any)*
Weizmann Institute
NCI:SB

LAB/BRANCH
Diabetes Branch

SECTION
Receptors and Hormone Action Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	2.9	2.1

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Multiple different biochemical defects have been identified in patients with genetic forms of extreme insulin resistance. These defects have included: (1) a decreased number of insulin receptors, (2) qualitatively abnormal insulin receptors which are impaired in their ability to couple insulin binding to insulin action; and (3) post-receptor defects in insulin action.

Cultured Epstein-Barr virus-transformed lymphocytes from insulin resistant patients have been used to study biosynthesis and turnover of insulin receptors. In those patients with a decreased number of insulin receptors there is a marked (∿90%) decrease in receptor biosynthesis with only a slight increase in the rate of receptor degradation.

In other patients, whose circulating monocytes possess a normal number of insulin receptors despite hyperinsulinemia, it was not clear why the hyperinsulinemia did not lead to down-regulation of receptors. However in cultured EBV-transformed lymphocytes from these patients down regulation of their insulin receptor could be demonstrated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 47024-05 DB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biosynthetic Labeling of the Insulin Receptor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	J. A. Hedo	Visiting Associate	DB, NIADDK
Others:	I. A. Simpson	Visiting Scientist	CMOS, NIADDK
	S. Cushman	Chief	CMOS, NIADDK
	M. Kasuga	Research Fellow	Boston, MA
	C. R. Kahn	Research Director	Boston, MA

COOPERATING UNITS *(if any)*

Cellular Metabolism and Obesity Section, NIADDK; Joslin Diabetes Center, Boston, Massachusetts

LAB/BRANCH

Diabetes Branch

SECTION

Cellular and Molecular Physiology Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies involving the biosynthesis and other aspects of the life cycle of the insulin receptor in human IM-9 lymphocytes as well as in isolated rat adipose cells are under investigation. The receptors are labeled by either biosynthetic incorporation of radioactive sugars and amino acids or by techniques that label proteins or glycoproteins at the cell surface. After labeling the receptors are isolated by immunoprecipitation with anti-receptor antibodies and analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis. In addition in order to characterize the subcellular sites responsible for the biosynthetic as well as for other processes of the life cycle of the receptor, three different subcellular fractions were prepared from rat adipose cells: 1) plasma membrane fraction; 2) high density microsomal fraction (enriched in endoplasmic reticulum; 3) low density microsomal fraction (enriched in Golgi apparatus. These results indicate that the insulin receptor is synthesized in the endoplasmic reticulum as a M_r = 190,000 high mannose precursor. This precursor exhibits functional properties such as the ability to bind insulin and to undergo autophosphorylation in response to insulin. Processing of the precursor to yield the major subunits M_r = 135,000 and 95,000. In addition, some of the precursor appears to escape proteolytic cleavage but undergoes full processing of the carbohydrate chains and generates the M_r = 210,000 component which is inserted in the plasma membrane.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 47025-01 DB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Tissue Insulin-Like Peptides and Embryo Receptors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	M. A. Lesniak	Chemist	DB, NIADDK
Others:	J. Grizard	Visiting Associate	DB, NIADDK
	S. A. Hendricks	Guest Worker	DB, NIADDK
	L. Bassas	Guest Worker	DB, NIADDK
	C. B. Hart	Guest Worker	DB, NIADDK
	M. Quarum	Bio Lab Tech	DB, NIADDK
	C.-D. Agardh	Visiting Associate	DB, NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Diabetes Branch

SECTION
Receptors and Hormone Action Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	4.5	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Additional studies in extracts of tissues of rodents, Sprague-Dawley rats, ob/ob mice and their thin littermates, control and diabetic Chinese hamsters, have confirmed our previous findings of the presence of an insulin-like material. Although brain and liver membrane receptors are influenced by circulating plasma insulin, the insulin-like tissue content of the same tissues are not influenced.

The status demonstrating the presence of insulin receptors in rodent brain and liver membranes were extended to chicken embryos. Insulin receptors are present in membrane preparations of heads and bodies of chick embryo by 4 days of incubation. Binding of labeled insulin to brain and liver membranes from 8 to 18 day embryos increases with time of development. Recently, insulin-like growth factor binding was studied in chick embryo tissue. Using labeled multiplication stimulating activity peptide there appears to be specific insulin-like growth factor receptors on brain membrane preparations of embryos as early as day 2 of development. The growth factor finding pattern is different than that for insulin binding in chick embryo.

Insulin receptor structure studies have been extended in rat brain and rat brain microvessels. The gel electrophoresis data indicate that the insulin receptor in whole brain homogenates is distinguished from those in peripheral tissues by structural alterations including changes in the carbohydrate moiety of the receptor. The microvessel insulin receptor exhibits features typical of liver membrane and atypical of whole brain homogenates. Microvessel and liver membrane preparations from rats made diabetic with streptozotocin showed changes in receptor number (up regulation) which is in contrast with whole brain receptors which showed no change.

Annual Report of the Pediatric Metabolism Branch, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases

During the period FY'1984 covered by this report, the Pediatric Metabolism Branch has undergone a transition due to the retirement of Dr. Paul di Sant'Agnese, the longtime Chief of this highly productive branch. The research activities of the branch have been redistributed to other branches within the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases and will be reported on as individual activities within those branches.

The clinical program continues to operate efficiently and effectively under the directorship of the Clinical Director, NIADDK. Dr. Milica Chernick directs a full-time clinic and in-patient service available for our cystic fibrosis patients. This program is prepared to provide clinical material to the various investigative groups in the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases when needs arise.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 49001-11PMB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Studies in Cystic Fibrosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Paul A. di Sant'Agnese, M.D.	Chief, PMB, NIADDK	
OTHER:	Van S. Hubbard, M.D., Ph.D.	Senior Surgeon	PMB, NIADDK
	Milica S. Chernick, M.D.	Staff Fellow	PMB, NIADDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Pediatric Metabolism Branch

SECTION

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0		

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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TERMINATE

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 49004-05PMB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Fatty Acids in Cystic Fibrosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Van S. Hubbard, M.D., Ph.D., Senior Surgeon, PMB, NIADDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Pediatric Metabolism Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 0	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 49005-05PMB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Follow-up of hypochloremic infant formula feedings

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Van S. Hubbard, M.D., Ph.D., Senior Surgeon, PMB, NIADDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Pediatric Metabolism Branch

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NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 0	PROFESSIONAL:	OTHER:

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 49007-03PMB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Fat Cell as a Model in Cystic Fibrosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Milica Spasovska-Chernick, M.D. — Staff Fellow, PMB, NIADDK

Marta Ulane, M.S. — Guest Worker, PMB, NIADDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Pediatric Metabolism Branch

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INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0		

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 49008-02PMB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Membrane Studies in CF

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Van S. Hubbard, M.D., Ph.D.,	Senior Surgeon, PMB, NIADDK
	Marta Ulane, M.S.,	Guest Worker, PMB, NIADDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Pediatric Metabolism Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 0	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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TERMINATE

ANNUAL REPORT OF THE CLINICAL HEMATOLOGY BRANCH

NATIONAL INSTITUTE OF ARTHRITIS, DIABETES, AND DIGESTIVE AND KIDNEY DISEASES

I. Study of Immunology of Blood Cell Deficiencies

A. Platelet Debris Containing Non-Specifically Adsorbed IgG and Other Membrane Markers is an Index of Platelet Destruction

Platelet-associated IgG as currently measured in immune thrombocytopenias does not appear to represent antiplatelet antibody because the increases are usually too high, change too rapidly, do not correlate with serum antibody and occur in certain non-immune diseases. We recently reported that circulating platelet debris resulting from platelet destruction can be detected by membrane markers, counts of particles $<2\mu m^3$, as well as by EM. This membranous debris with adsorbed nonspecific IgG can account for elevated PAIgG in platelet suspensions. The debris is variably recovered at 2,000g sedimentation but quantitatively recovered at 30,000g. Pellets made at 30,000g from PPP of patients with destructive platelet disorders contained up to 8-fold the IgG of platelets sedimented at the usual 2,000g from an equal volume of PRP whereas similar PPP-pellets from controls contained only 0.2 the IgG of platelets from PRP. Assays of platelet membrane markers on PPP-pellets may more accurately assess platelet fragmentation than PAIgG assays on platelet suspensions. We conclude that platelet debris is a hallmark of rapid platelet turnover, accounts for the discrepant observations on PAIgG and may contribute to apparent increased hemostatic effectiveness of platelets in destructive disorders.

B. IgA and IgG are Present in Equal Amounts in Normal Platelets, IgM in Lesser Amounts, and all Three Ig Classes are Elevated in Destructive Thrombocytopenias

A new quantitative acid elution technique and solid phase RIA were developed to accurately measure immunoglobulins associated with platelets. Platelets, washed 4 times and suspended in saline, were brought to pH 2.9 with 50 mM acetic acid containing 100 mM NaCl and 0.5% BSA. After 10 min, the supernatant of centrifuged platelets was brought to pH 8.1 with Tris base and added to microtiter wells coated with heavy chain specific affinity purified antibody. After 2 hours of incubation, wells were washed, then incubated for 1-1/2 hrs. with ^{125}I-labeled antibody identical to the coating antibody, washed again and counted. Logit transformation of the data gave linear curves with IgG, IgA, IgM and IgE from 0.5 to 50 ng. The amount of Ig eluted was linear with respect to platelet concentration. Acid exposure did not decrease reactivity of Ig more than 10% and antibodies against the different Ig classes did not significantly cross react. Sequential acid elutions of the same platelet pellet released additional but decreasing quantities of Ig. On the first elution, 64% of the IgG was released compared to 27% IgA and 85% IgM, indicating that IgA is the most tightly bound and IgM the least. Values for total amounts of the different Ig classes obtained from normal platelets by 4 sequential elutions, expressed as fg/platelet (n=7), were: IgG, 2.01±0.13; IgA, 2.00±0.04; IgM, 0.06±0.007; IgE, undetectable. Patients with ITP and Wiscott-Aldrich syndrome whose platelet-associated IgG (PAIgG) was high also had high PAIgA and PAIgM. Although previous studies of PAIg focus almost exclusively on IgG, our observations indicate that, in normal platelets, the IgA:IgG ratio is markedly increased over that of plasma, and IgM is present by sensitive assay and the IgM:IgG radio is similar to that of plasma.

All 3 Ig classes are elevated in destructive thrombocytopenias.

C. An *In Vivo* Model Demonstrating that Elevated Platelet-Associated IgG is a Non-Specific Consequence of Platelet Destruction

We recently reported that circulating platelet debris accompanies elevated PAIgG in patients with diseases causing platelet destruction. Nonspecific IgG associated with this debris appeared to account for many discrepant observations on PAIgG unexplained by the tenet that elevated PAIgG is due to antiplatelet antibodies. We produced thrombocytopenia (TP) in dogs with i.v. rabbit anti-dog platelet antibodies and measured platelet-associated dog IgG (PA DIgG) and albumin, using rabbit anti-dog IgG in a C-lysis inhibition system (CLI) and by RIA. During induction of TP (~ 2000 pl./µl) PA DIgG rose as high as 65-fold above the normal values of 1.5±0.5 fg/pl. Concomitant with a rise in PAIgG there was an equivalent rise in platelet-associated albumin. This was synchronous with appearance of a peak of debris <0.9 µm^3 in PRP. The PA DIgG returned to normal in 3 days as debris disappeared; platelet counts and size returned to normal by the 6th day. The amount of rabbit anti-dog platelet IgG used to induce TP, if distributed evenly on platelets *in vivo*, would have provided no more than 0.1 to 0.4 fg rabbit IgG/pl. and was not detectable by CLI with goat anti-rabbit IgG. Thus, thrombocytopenia induced by amounts of antibody too low to detect produced very high levels of PAIgG. This model reproduces observations on clinical immune thrombocytopenias in which high levels of PAIgG (>100 fg/pl.) grossly exceeded the highest saturating amounts of (~ 2 fg/pl.) of known human antiplatelet antibodies. Although we find circulating platelet debris (<2 µm^3) in all destructive platelet disorders, the estimated quantity may be insufficient to account for PAIgG levels simply on the basis of normal adsorption of nonspecific IgG to excess membrane. Much platelet debris on EM consists of membranes devoid of cytoplasm, which may re-seal and encompass plasma. Since plasma proteins contained in a volume equivalent to 1 platelet would contain 100-fold the amount of IgG in a normal platelet, resealing may account in part for PAIgG and for platelet-association of plasma proteins other than IgG.

D. Elevated Platelet-Associated IgG in SLE is not Associated with Increased Platelet Turnover

The accepted concept that almost all nonthrombocytopenic SLE patients have increased platelet turnover with a "compensated thrombocytolytic state" is based on a study in which 5 of 5 patients had shortened platelet survival using a DF^{32}P label and 6 of 8 patients had megathrombocytosis presumed to indicate increased platelet production. Positive indirect PF3 or serotonin release tests in >50% of SLE patients, and more recent reports that approximately 50% have elevated platelet-associated IgG (PAIgG) seem to support this concept. In a group of 78 SLE patients with platelet counts >130,000/µl, we found 23 (29%) with elevated PAIgG by C-lysis inhibition and RIA techniques. Elevated values on SLE patients were within 5-15 fg/platelet (normal 0.5-2.5 fg/platelet) with one exceptionally high value of 56 fg. PAIgG levels did not correlate with the severity or duration of SLE and 5 patients with PAIgG (including the 56 fg value), when retested as soon as 3 weeks later, showed normal values without change in platelet count or clinical status. Survival studies using ^{51}Cr labeled autologous platelets, performed on 6 SLE patients, 3 with normal PAIgG (1-1.5 fg/platelet) and 3 with elevated PAIgG (7-10 fg/platelet) all gave normal values. Megathrombocytes defined as having a volume by the Coulter Channelizer equal to or > the upper 10% of normal platelets, were not increased in 8 of 8 SLE patients.

Additionally there was no electron micrographic evidence of platelet fragmentation in SLE patients in contrast to ITP patients whose high PAIgG levels appear to be attributable to nonspecific IgG associated with circulating platelet debris (see above). Although tests for immune complexes did not correlate with elevated PAIgG or serotonin release, these positive serologic tests with platelets in SLE may reflect the presence of antigen-antibody complexes that frequently accompany this disease, but are not uniformly measurable. None of the tests predict in vivo platelet injury or survival.

E. A Sensitive Method for Detecting Specific Anti-Platelet Antibodies of Clinical Significance

We have found in our studies of over 200 cases of isoimmune neonatal thrombocytopenia (INT) that approximately 70% of the cases are due to maternal immunization against the Pl^{A1} antigen. This is determined by phenotyping maternal and paternal platelets. Since only 2% of the population lacks the Pl^{A1} antigen, finding that the mother of a thrombocytopenic infant is Pl^{A1}-negative and the father Pl^{A1}-positive is strong presumptive evidence for INT. Phenotypic incompatibility in the Pl^{A} system has been found in approximately 70% of suspected cases of INT; but, until now, could not be confirmed by demonstration of maternal serum antibodies because the responsible antibodies do not fix complement with or aggregate platelets and rarely are detectable by "sandwich" radioimmunoassay. However, we found that positive serologic tests could be obtained if platelet membrane antigens were separated by the acrylamide gel-introcellulose "blotting" technique, exposed to maternal serum, and developed with radiolabeled anti-human IgG and photography. Maternal sera that are positive for antibody produce a dark line corresponding to the electrophoretic position of the platelet antigen. Sera from approximately 90% of mothers with Pl^{A1}-negative phenotype whose infants wer thrombocytopenic, produced a dark line at the level of platelet glycoprotein I which contains the Pl^{A1} antigen. Thus, approximately 60% of suspected cases of INT can be diagnosed in this manner compared to approximately 5% by serologic techniques used previously. The test appears to be applicable in predicting whether future pregnancies will be complicated by INT.

The same "blotting" technique has been used to diagnose cases of post-transfusion purpura (PTP). Of 25 cases of PTP, sera from 90% were positive. Of special interest was the finding that antibodies responsible for PTP were not limited to anti-Pl^{A1} as previously supposed for an antigen in a clearly different electrophoretic position reacted with sera from a classic case of PTP. This new antigen is being studied with respect to its molecular structure and characteristics.

F. Serology of New Platelet Antigens

In the course of studying 22 cases of INT and 6 cases of PTP this year, we found that antibodies responsible for the disease in 1 case of INT and 3 cases of PTP had specificities differinf from those previously identified. The antibody from one case of PTP clearly identifies a platelet membrane component separable by electrophoresis and solid phase lectin columns. The molecular characteristics of this antigen are under study. Antibody from another case of INT and one case of PTP has a specificity that appears to identify a third allele in the Pl^{A} system which until now has been clearly bi-allelic. Three of the antibodies cross react with lymphocytes and would be categorized as anti-HLA antibodies, but one of these reacts with a platelet membrane antigen of molecular size much too large to be an HLA antigen of the type that has been structurally identified in lymphocytes. Work is in progress to resolve these seeming paradoxical findings.

II. Study of Blood Coagulation and Diseases of Hemorrhage and Thrombosis

A. Defibrination by Thrombin Without Causing Thrombocytopenia

Thrombocytopenia has been considered a hallmark of disseminated intravascular coagulation (DIC). Because thrombin is one of the most potent platelet aggregating agents in vitro and because platelets adhere firmly to fibrin meshes, the general assumption has been that the thrombocytopenia of DIC is owing to the aggregation of platelets by thrombin exacerbated by entrapment of platelets in clots. We infused dogs with purified thrombin in attempts to produce the thrombocytopenia of DIC in order to study the effects of DIC on platelet sequestration. Surprisingly, thrombin infusions sufficient to cause complete in vivo defibrination did not decrease the platelet count. Infusion of additional thrombin in amounts 10-fold more than necessary to cause maximum in vitro platelet aggregation and release had no apparent effects on platelets in vivo. These observations raise the possibilities that a crucial cofactor for platelet aggregation (perhaps fibrinogen) is depleted early in the course of thrombin infusion, that in vitro observations are not relevant to effects of thrombin in vivo, that DIC leads to thrombocytopenia by mechanisms other than the action of thrombin, or that the rate of thrombin formation rather than thrombin formation per se is the determinant factor in producing thrombocytopenia. Studies to evaluate these and other questions raised by this provocative observation are in progress.

B. Platelet abnormalities in Bartter's Syndrome

Bartter's syndrome consists of a group of abnormalities including increased circulating angiotensin, aldosteronism, hypokalemic alkalosis, hypomagnesmia, hyperuricemia, increased urinary prostaglandin E_2, and other abnormalities. Recently it has been found that platelets of these patients respond poorly to aggregating agents, particularly to ADP despite otherwise normal platelet function. In six cases of Bartter's syndrome we found impaired platelet aggregation to ADP that appeared to be proportional to the severity of the disease and to revert toward normal with therapy that elevated serum potassium. On electronmicroscopy, platelets of 2 of the patients with markedly impaired aggregation showed marked cytoplasmic vacuolization whereas platelets from less severely affected patients were indistinguishable from normal. Further work is in progress to evaluate these findings.

C. Effects of a Meperidine Analog that Causes Parkinsonism on Physiologic Responses of Cultured Chromaffin Cells

N-methyl-4-phenyltetrahydropyridine (NMPTP) has been shown to cause a neurological lesion which is indistinguishable from Parkinsonism in humans and primates. However, the mechanism of this toxicity is unknown. The effects of NMPTP and various analogs were studied on primary cultures of bovine adrenal chromaffin cells. (BACC). The long-term toxicity did not appear to be striking but early studies showed that NMPTP was a very effective catecholamine releasing agent. The acute pharmacology was studied and it was determined that NMPTP was a potent cholinergic agonist. NMPTP release of catecholamine is calcium dependent and is blocked by ganglionic blockers. Binding studies showed that a high affinity, saturable binding site is present. Specific binding is displaced by nicotine and lobeline. This may be an important clue to the mechanism of action of NMPTP in precipitating Parkinson's disease.

D. Regulation of Peptide Secretion in Cultured Chromaffin Cells

Met-enkephalin (Menk) is present in considerable quantities in the adrenal medulla and is the largest single source of peripheral Menk. We are studying mechanisms for regulation of the translation of the Menk gene, processing of the messenger RNA and translation of the message into protein. Reserpine has been shown by others to increase Menk; we have shown that this is not primarily due to an increase in Menk mRNA, since there is no dramatic change in Menk mRNA. However, Menk mRNA does increase dramatically with increases in intracellular cyclic AMP. We propose that this model supports at least two modes of regulation of Menk; one is at the level of transcription and is enhanced by cAMP, the other is probably at the level of translation and, in the adrenal chromaffin cell, can be enhanced by reserpine.

Vasoactive intestinal polypeptide (VIP) has been shown by us to appear during culture of bovine adrenal chromaffin cells. We have studied the mechanism of this change and evaluated the distribution of VIP in these cells by immunofluoresence techniques. Increases in intracellular cAMP causes 3- to 10-fold increases in VIP and we propose that this is a key regulatory process in the appearance of VIP in cultures. Immunofluorescence studies show that VIP is only located in 10 to 15% of cells in the culture, in contrast to Menk which is present in 80% of cells. Catecholamines are also found in about 80% of these cells; 10 to 15% of these cells are fibroblast-like and do not contain catecholamine VIP or enkephalin. Therefore, we conclude that a subpopulation of chromaffin cells changes their state of differentiation in response to the change from _in vivo_ to _in vitro_ and that the signal is probably transduced to the cell's biochemical systems by an adenylate cyclase mediated process.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 51,001-26 CHB

PERIOD COVERED
Ocober 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of Blood Coagulation and Diseases of Hemorrhage and Thrombosis

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
N. Raphael Shulman Chief CHB NIADDK

COOPERATING UNITS *(if any)*
Hypertension Endocrine Branch, NHLBI
Laboratory of Clinical Science, NIMH
Clinical Neuroscience, NIMH

LAB/BRANCH
Clinical Hematology Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.75	1.5	0.25

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Stimuli causing aggregation of platelets and their secretory release reaction are the most likely causes of thrombosis. Thrombin is the most powerful in vitro platelet stimulus thought to underlie in vivo thrombotic phenomena and to cause thrombocytopenia of disseminated intravascular clotting. We found that dogs given intravenous thrombin in variable doses could be completely defibrinated without causing thrombocytopenia platelet aggregation or thrombus formation, indicating that factors other than initiation of blood coagulation underlie thrombosis.

Platelets in Bartter's syndrome were found to be poorly responsive to ADP aggregation and on EM appear to have abnormal vacuolization that may vary with electrolyte balance.

Platelet secretion has many features in common with chromaffin cell secretion of catecholamines vasoactive intestinal polypeptide (VIP) and enkephalin; and both types of cells respond similarly to certain secretory agonists and inhibitors. Cultured chromaffin cells have been used to measure regulation of peptide secretion with respect to changes in transcription and translation utilizing mRNA and cDNA to evaluate effects of secretory stimuli and inhibitors on message formation. Tripeptide aldehyde protease inhibitors which inhibit thrombin were found to block catecholamine release in a manner similar to their inhibition of platelet release. A meperidine analog NMPTP, which causes a syndrome identical to Parkinsonism was found to kill cultured chromaffin cells over several days and to acutely cause release of catecholamines.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 51,000-26 CHB

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of Immunology of Blood Cell Deficiencies

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
N. Raphael Shulman Chief CHB NIADDK

COOPERATING UNITS *(if any)*
Arthritis and Rheumatism Branch, NIADDK
Neuroendocrine Branch, NIMH

LAB/BRANCH
Clinical Hematology Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.25	1.5	0.75

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The immunologic disorders, idiopathic thrombocytopenic purpura (ITP), systemic lupus erythematosus (SLE) neonatal(INT)-post-transfusion(PTP)-and drug-purpura account for the majority of clinical thrombocytopenias. The immune reactions involved in these disorders are relevant to understanding autoimmunity, histocompatibility, malignant surveillance, isoimmune reactions interfering with support of chemotherapy and cellular immune injury generally. We have developed more precise and sensitive tests for cell-associated IgG,IgM,IgA and albumin and found that immune injury by specific antibody causes platelets to associate with nonspecific Ig's and albumin. By electronmicroscopy this association appears to result from trapping of plasma protein by cytoplasmic-free platelet membranes rather than by association of Ig's with Fc receptor. An animal model for ITP was developed that duplicated the cellular and immunologic abnormalities of this disease. Platelet immune abnormalities of SLE were found to differ from those of ITP in that cell-associated Ig's appeared to be due to immune complexes rather than nonspecific entrapment of plasma. Four cases of PTP were found to be due to isoantibodies to previously unrecognized platelet antigen specificities; we have extensively determined the molecular characteristics of one of these determinants. We have developed a intracellulose transfer technique to separate platelet membrane proteins that permits detecting antiplatelet antibodies of INT and PTP more sensitively and specifically than previously possible. Methods were developed to purify platelet membrane glycoproteins to obtain large amounts for clinical diagnostic tests.

ANNUAL REPORT SUMMARY OF THE GENETICS AND BIOCHEMISTRY BRANCH

NATIONAL INSTITUTE OF ARTHRITIS, DIABETES, AND DIGESTIVE AND KIDNEY DISEASES

Molecular Genetics Section

Dr. Camerini-Otero and members of his section have shown that when foreign DNA is introduced into mouse Ltk$^-$ cells and the DNA is integrated into the host genome the foreign sesquences are flanked by moderately repetitive sequences. The integration is accompanied by deletions or rearrangements of the mouse DNA.

Transfection of mouse Ltk$^-$ cells has also been used by Dr. Camerini-Otero and his colleagues to study homologous recombination in somatic cells in culture. They have shown that when non-overlapping mutants of the herpes simplex virus thymidine kinase genes are cotransferred into mouse Ltk$^-$ cells they give rise to recombinants responsible for the tk$^+$ phenotype. The recombinants were shown to arise as a result of homologous pairing and strand exchange between the two mutant sequences and not as a consequence of either ligations or single-strand annealing of the original substrate DNAs. It was shown that non-homologous inserts and deletions are handled differently by the recombination machinery, that is there is a donor-recipient asymmetry in the recombination process.

Recently, a cell-free system for recombination has been developed. The substrates for this reaction are two mutants of the tetracycline resistance gene of _E. coli_. Incubation of these two mutants with partially purified nuclear extracts from mammalian cells reconstructs the wild type gene. This assay is being used to dissect the recombination machinery and to investigate the possible role of recombination in certain human diseases such as Bloom's syndrome, ataxia telangiectasia and xeroderma pigmentosa.

Biochemical Genetics Section

Dr. A. Robbins has shown that a single genetic lesion affects Golgi function, endocytosis and ATP-dependent endosomal acidification in Chinese hamster ovary cells. Her results suggest that both Golgi and endosomes are dependent on a common component for acidification and that Golgi acidification may be required for some steps in post-translational modification of glycoproteins.

Dr. Neufeld and her colleagues have extended studies of β-hexosaminidase synthesis. A cDNA fragment encoding the α-chain of the enzyme was obtained by the strategy of 1) purification of mRNA from normal human fibroblasts by immunoselection, and 2) cloning by the primer-vector procedure of Okayama and Berg. Northern blot analysis using this cDNA showed a lack of the complementary mRNA in fibroblasts of patients with classic Tay-Sachs disease who are of Ashkenazi origin. Isolation and characterization of full-length cDNA and of genomic DNA have been undertaken to determine the precise nature of the mutation.

In collaboration with investigators in the National Institute of Child

Health and Development a protocol has been initiated to test the value of amnion implants in the treatment of mucopolysaccharide storage disorders. Th rationale is that the implants would provide lysosomal enzymes to the recipients. Preliminary results include positive subjective changes reported by parents but no change in serum or leukocyte enzyme levels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 52000-17 GBB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Biochemical Lesions in Genetic Disorders of Lysosomal Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. F. Neufeld	Chief	GBB, NIADDK
Others:	R. Myerowitz	Staff Fellow	GBB, NIADDK
	R. Proia	Staff Fellow	GBB, NIADDK
	G. Sahagian	Staff Fellow	GBB, NIADDK
	A. d'Azzo	Visiting Fellow	GBB, NIADDK
	S. Laurie	Visiting Fellow	GBB, NIADDK
	L. Poenaru	Visiting Scientist	GBB, NIADDK
	C. Hall	Research Chemist	GBB, NIADDK
	J. Muenzer	Medical Staff Fellow	HGB, NICHD

COOPERATING UNITS *(if any)*

Human Genetics Branch, NICHD, NINDS
Department of Biological Chemistry, UCLA

LAB/BRANCH

Genetics and Biochemistry Branch

SECTION

Biochemical Genetics Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.6	7.2	1.4

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Tay-Sachs disease is a lysosomal storage disorder caused by mutation of the gene encoding the alpha chain of beta-hexosaminidase A. Cultured fibroblasts derived from some patients fail to synthesize any protein corresponding to the alpha chain, whereas those of others synthesize cross-reactive material (CRM) that does not form competent enzyme. Previously, we had shown that fibroblasts of late infantile Tay-Sachs patients of Ashkenazi origin are CRM-negative due to absence of functional mRNA. We have cloned a cDNA fragment corresponding to the alpha chain and shown absence of mRNA by Northern blot analysis in cells of such patients. Isolation and sequencing of full-length cDNA and of genome clones has been undertaken to determine the precise nature of the mutation.

Similar studies have been undertaken with the beta chain of beta-hexosaminidase; cells of several patients with beta chain defects (Sandhoff disease) have likewise shown the existence of CRM-positive and CRM-negative mutations.

In collaboration with investigators at NICHD, a protocol has been initiated to test the clinical value of amnion implants in the treatment of mucopolysaccharide storage disorders. The rationale is that the implants would provide lysosomal enzymes to the recipients. Preliminary results include subjective changes reported by parents but no change in serum or leukocyte enzyme levels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 52002-07 GBB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Role of Cell-Surface Antigens in Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.D. Camerini-Otero	Acting Chief	GBB, NIADDK
Others:	D.A. Brenner	Medical Staff Fellow	GBB, NIADDK
	J. Silverman	Medical Staff Fellow	ARB, NIADDK
	J. Klippel	Senior Investigator	ARB, NIADDK
	D. Margulies	Medical Staff Fellow	LMG, NICHD
	G. Evans	Medical Staff Fellow	LMG, NICHD
	E. Appella	Acting Chief, Chemistry Sect.	LCB, NCI
	J. Seidman	Asst. Professor, Dept. of Genetics Harvard Medical School	

COOPERATING UNITS *(if any)*

Arthritis and Rheumatism Branch, NIADDK; Dept. of Genetics, Harvard Med. School
Laboratory of Molecular Genetics, NICHD
Laboratory of Cell Biology, NCI

LAB/BRANCH

Genetics and Biochemistry Branch

SECTION

Molecular Genetics Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATE

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 52008-05 GBB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Gene Expression and Human Genetics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. D. Camerini-Otero	Acting Chief	GBB, NIADDK
Others:	S. Kato	Visiting Associate	GBB, NIADDK
	D. A. Brenner	Medical Staff Fellow	GBB, NIADDK
	D. Daugherty	Medical Staff Fellow	GBB, NIADDK
	S. M. Meyn	Medical Staff Fellow	CC
	M. Hsieh-McIntosh	Staff Fellow	GBB, NIADDK
	A. Smigocki	Microbiologist	GBB, NIADDK
	C. Camerini-Otero	Medical Staff Fellow	GBB, NIADDK
	F. Mills	Guest Worker	GBB, NIADDK

COOPERATING UNITS *(if any)*

NIH Interinstitute Medical Genetics Program
Laboratory of Molecular Biology, NIADDK
Department of Medicine, University of Iowa School of Medicine

LAB/BRANCH

Genetics and Biochemistry Branch

SECTION

Molecular Genetics

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.1	7.7	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have used DNA-mediated gene transfer to study gene expression and recombination in in the cells of higher eukaryotes. In one series of experiments the thymidine kinase gene of herpes simplex virus I (tk) and pBR322 were introduced in mouse Ltk- cells by the calcium phosphate method. We have subsequently cloned, sequenced and characterized seven junctions between the foreign DNA and the host genome. In all cases the insertions have occurred into the repetitive components of the mouse genome. We have extended our investigations beyond the study of nonhomologous recombination mentioned above to the study of homologous recombination. In this study we have used a family of plasmids with insertion and deletion mutations in the coding region of tk as substrates for intermolecular recombination events. There were three major findings of these studies. First, we were able to show for the first time that the tk+ phenotype that was generated by the cotransfer of two tk mutants was the result of recombination and not complementation at the protein level. This was accomplished by cloning by the technique of plasmid rescue the sequences responsible for this phenotype. Second, using a novel experimental paradigm we were able to demonstrate that these recombinants were the result of true homologous pairing between the molecules bearing the mutations and not the result of ligations between the molecules bearing the mutations (cut and paste models) or of single-strand annealing between molecules appropriately processed by exonucleases. Third, that there is a sharp discontinuity in the manner in which insertions are handled by the recombination machinery depending on whether they are smaller or larger than 24 base pairs. Most recently, we have developed a cell-free recombination system that utilizes partially purified nuclear extracts from mammalian cells. We are also using this cell-free system to screen extracts from human diploid fibroblasts of patients with diseases such as Bloom's syndrome, ataxia telangiectasia, and xeroderma pigmentosa to determine whether these conditions involve primary or secondary defects in some of the proteins mediating recombination.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 52009-05 GBB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Endocytosis, Secretion and Compartmentalization in Mutant CHO Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. R. Robbins	Research Geneticist	GBB, NIADDK
Others:	C. W. Hall	Research Chemist	GBB, NIADDK
	C. F. Roff	Staff Fellow	GBB, NIADDK
	J. L. Bateman	Biologist	GBB, NIADDK
	C. Oliver	Research Biologist	LBS, NIDR
	I. Mellman	Asst. Professor, Yale Univ. School of Medicine	
	S. S. Krag	Assoc. Prof., Johns Hopkins Univ. School of Public Health	

COOPERATING UNITS *(if any)*

Laboratory of Biological Structure, NIDR
Department of Cell Biology, Yale University School of Medicine
Department of Biochemistry, School of Public Health, Johns Hopkins University

LAB/BRANCH

Genetics and Biochemistry Branch

SECTION

Biochemical Genetics Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.7	2.5	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have shown a severe decrease in ATP-dependent acidification of endosomes isolated from a Chinese hamster ovary cell mutant pleiotropically defective in receptor-mediated endocytosis. This mutant DTG 1-5-4, and many other of our endocytosis mutants, also exhibited loss of some Golgi-associated functions: in DTG 1-5-4 asparagine-linked oligosaccharides on Sindbis virus glycoproteins were not galactosylated and Sindbis virus assembled on Golgi cisternae rather than on the plasma membrane; also, some endogenous glycoproteins showed incomplete terminal glycosylation. Completion of glycosylation in the mutant seems dependent on the nature of the glycoprotein acceptor: glycoprotein G of vesicular stomatitis virus was galactosylated although Sindbis glycoproteins were not; most cell-associated glycoproteins were glycosylated normally but secreted glycoproteins were not. In DTG 1-5-4 defects in endocytosis, endosomal acidification and Golgi function result from a single genetic lesion since these activities were all restored in revertants of DTG 1-5-4 and on genetic complementation.

To determine if impairment of Golgi-associated post-translational modification in the mutants resulted secondarily in defective endocytosis, we have examined a temperature-sensitive mutant. After a shift to non-permissive temperature (39 C) endocytic activities were lost prior to detectable changes in post-translational modification. Also, endosomes isolated from the mutant grown at the permissive temperature (34 C) exhibited ATP-dependent acidification at 34 C, but not at 39 C. Thus, the endosomal defect is not secondary to the Golgi defect; we suggest that endosomes and Golgi require a common component, defective in the mutants. We are presently attempting to identify this component.

Annual Report of the Digestive Diseases Branch
National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases

SUMMARY OF BRANCH ACTIVITIES

The Digestive Diseases Branch has two sections (Section on Gastroenterology and the Liver Diseases Section). The Liver Diseases Section has 2 senior physicians and 3 medical staff fellows; the Section on Gastroenterology has 3 senior physicians and 5 medical staff fellows. The Digestive Diseases Branch also has 5-10 guest investigators from other laboratories.

Detailed summaries of the activities of each section precede the individual project reports. Both sections are engaged in investigations of basic biologic processes (e.g., hormone action, membrane transport, cellular and humoral immunology) and are attempting to apply this information to understand the pathophysiology of various disorders involving the liver and gastrointestinal tract. Both sections are also involved in attempts to improve therapy of clinical disorders such as neoplasms associated with overproduction of gastrointestinal hormones, hepatitis and fulminent hepatic failure.

Section on Gastroenterology

We are in the process of following more than 70 patients with Zollinger-Ellison syndrome (ZES, gastrin-producing neoplasm, hypergastrinemia and increased secretion of gastric acid). All patients are currently being managed using oral medication and none has required a total gastrectomy. During the past three years we have found that cimetidine and ranitidine are equally effective in inhibiting gastric acid secretion in patients with ZES. One problem that occurs with cimetidine but not ranitidine is that approximately 50% of male subjects who take doses of cimetidine sufficient to control their gastric acid secretion will develop impotence. Patients who require high doses of cimetidine to control their gastric acid secretion require proportionately high doses of ranitidine. Presently we are evaluating the role of surgery in patients with ZES. In particular we are attempting to localize and resect the gastrinoma with the hope that the patient can be cured of ZES. We are evaluating 2 new gastric antisecretory agents, famotidine and omeprazole, in patients with ZES.

It has been known for some time that tetraethylammonium can inhibit pancreatic enzyme secretion. During this past year we have found that tetraethylammonium inhibits pancreatic enzyme secretion by functioning as a muscarinic cholinergic receptor antagonist. In particular, in dispersed acini from guinea pig pancreas, tetraethylammonium inhibits the stimulation of enzyme secretion caused by carbachol but does not alter the stimulation of secretion caused by other secretagogues such as cholecystokinin, bombesin, physalaemin, VIP or secretin. There is a close correlation between the ability of tetraethylammonium to inhibit carbachol-stimulated enzyme secretion and its ability to inhibit binding of [^{3}H]-N-methylscopolamine to muscarinic cholinergic receptors on pancreatic acini.

For several years natural glucagon has been reported to be capable of stimulating pancreatic enzyme secretion. We have found that the observed stimulation of pancreatic enzyme secretion is not attributable to glucagon *per se* but instead, to one or more peptides that contaminate the currently available preparations o natural glucagon. These contaminants which do not interact with any of the known secretagogue receptors on pancreatic acinar cells, stimulate enzyme secretion by causing activation of adenylate cyclase and increased cellular cyclic AMP. Purification and characterization of these glucagon-contaminants should permit the identification of a previously unrecognized class of secretagogue receptors on pancreatic acinar cells.

Dispersed acini from guinea pig pancreas possess two classes of receptors each of which interact with VIP and secretin. One class is VIP-preferring in that it has a high affinity for VIP and a low affinity for secretin. The other class is secretin-preferring in that it has a high affinity for secretin and a low affinity for VIP. Initially these two classes of receptors were identified by measuring binding of ^{125}I-VIP. Recently, however, we have found that the secretin-preferring receptors can be examined more clearly by measuring binding of ^{125}I-secretin. (Iodination of secretin is difficult and tedious, because the peptide possesses no tyrosine but does have an N-terminal histadine). Occupation of secretin-preferring receptors by an appropriate agonist causes activation of adenylate cyclase and a 100-fold increase in cellular cyclic AMP. There are "spare" secretin-preferring receptors in the sense that occupation of only a fraction of the receptors is sufficient to cause maximal activation of adenylate cyclase.

In contrast to acinar cells from guinea pig pancreas, with acinar cells from rat pancreas four classes of receptors are required to account for the actions of VIP and secretin on enzyme secretion, cellular cyclic AMP and binding of ^{125}I-VIP and ^{125}I-secretin. One class has a high affinity for VIP and occupati of this class of receptors causes increased cellular cyclic AMP and stimulation of enzyme secretion. A second class has a low affinity for VIP and for secretin, and occupation of these receptors does not cause changes in cyclic AMP or amylase secretion. A third class of receptors has a high affinity for secretin, and occupation of these receptors causes increased cyclic AMP and stimulation of enzyme secretion. A fourth class of receptors has a low affinity for secretin, and occupation of these receptors causes stimulation of enzyme secretion by a non-cyclic AMP-mediated mechanism.

Of the cells in the gastric mucosa, 25-30% are chief cells. To study gastric chief cell function one must develop a technique for isolating and enriching chief cells from gastric mucosa. During this past year we have found that one can prepare a suspension of isolated gastric mucosal cells containing over 90% chief cells by digesting gastric mucosa with collagenase followed by calcium chelation and then passing the cells over a Percoll density gradient. These chief cells show a 5-fold increase in the rate of pepsinogen secretion in response to secretagogues such as CCK, carbachol, secretin, VIP, 8Br-cyclic AMP or A23187. Secretion is temperature-dependent and requires intact oxidative metabolism.

Benzotript, N-p-chlorobenzoyl-L-tryptophan, is a specific CCK receptor antagonist. During this past year we used dispersed pancreatic acini to examine tryptophan as well as several different N-acyl derivatives of tryptophan for their abilities to function as CCK receptor antagonists. L-tryptophan, D-tryptophan as well as each acyl derivative tested inhibited CCK-stimulated amylase secretion and outflux of ^{45}Ca, and there was a close correlation between the ability of a particular agent to inhibit the action of CCK on acinar cell function and its ability to inhibit binding of ^{125}I-CCK to pancreatic acini. Results with butyloxycarboyl-L-trpytophan, indicated that the inhibition of the action of CCK caused by L-tryptophan and various acyl derivatives is specific, competitive and fully reversible. In functioning as a CCK receptor antagonist the relative potencies of the agents tested were: carbobenzoxy-L-tryptophan > benzotript > benzoyl-L-tryptophan = butyloxycarbonyl-L-tryptophan > acetyl-L-tryptophan > L-tryptophan. In inhibiting the actions of CCK native as well as N-acyl derivatives of D-tryptophan were equipotent with the corresponding compound containing L-tryptophan. These results demonstrate that the more hydrophobic the N-acyl moiety, the greater the affinity of the derivative for the CCK receptor.

Previously we have shown that C-terminal partial sequences of CCK function as CCK receptor antagonists. During this past year we synthesized CCK-26-32-NH$_2$, Ac-CCK-26-32-NH$_2$ and Ac-CCK-26-32. Each peptide inhibited the action of CCK on pancreatic acinar cells. Half-maximal inhibition of CCK-stimulated enzyme secretion occurred with 10 μM CCK-26-32-NH$_2$, with 25 μM Ac-CCK-26-32-NH$_2$ and with 100 μM Ac-CCK-26-32. Each peptide caused a parallel rightward shift in the dose-response curve for CCK-stimulated amylase secretion, and each peptide inhibited the stimulation of amylase secretion caused by CCK, caerulein and gastrin but did not alter the stimulation of enzyme secretion caused by bombesin, physalaemin, carbachol, A23187, secretin, VIP or 8Br-cyclic AMP. There was a close correlation between the abilities of derivatives of CCK-26-32 to inhibit binding of ^{125}I-CCK and their abilities to inhibit the stimulation of amylase secretion caused by CCK. These findings indicate that each of the three derivatives of CCK-26-32 is a specific competitive antagonist of the interaction of CCK with its cell surface receptors on pancreatic acini. The C-terminal amide appears to be an important determinant of the apparent affinity of the peptide for the CCK receptor whereas the N-terminal acetyl group appears to have little or no influence on the affinity of the peptide for the CCK receptor.

During this past year we examined the abilities of three analogues of substance P, [D-Pro^2, D-Phe^7, D-Trp^9]-substance P, [D-Pro^2, D-Trp^{7,9}]-substance P and [D-Arg^1, D-Pro^2, D-Trp^{7,9},Leu^{11}]-substance P, to alter substance P-induced changes in pancreatic acinar cell function and to occupy substance P receptors. Each peptide was able to inhibit the stimulation of amylase secretion caused by substance P receptor agonists. The inhibition was competitive, reversible and specific for peptides that interact with substance P receptors. For each analogue of substance P there was a close correlation between its ability to inhibit physalaemin-stimulated amylase release and its ability to inhibit binding of ^{125}I-physalaemin.

In the course of studying various substance P receptor antagonists, we found that [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]-substance P also functions as a bombesin receptor antagonist. In particular this analogue of substance P inhibits bombesin-stimulated amylase secretion and outflux of ^{45}Ca, and there is a close correlation between the ability of the analogue to inhibit the action of bombesin on acinar cell function and its ability to inhibit binding of ^{125}I-[Tyr^4] bombesin to pancreatic acinar cells. The inhibition of the actions of bombesin caused by [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]-substance P is competitive, reversible and specific for those secretagogues that interact with bombesin receptors.

Liver Diseases Section

The Liver Diseases Section is currently conducting seven principal studies:

I. Studies of the Pathogenesis of Encephalopathy in Fulminant Hepatic Failure

The patterns of visual evoked potentials in the central nervous system have been studied in rabbits. The recording of visual evoked potentials has been shown to be a reliable, quick, non-invasive method of quantifying neurologic changes during the evolution of hepatic encephalopathy (HE). Furthermore, the pattern of visual evoked potentials associated with HE is similar to that found in the postictal state and coma induced by barbiturates, benzodiazepines and the γ - aminobutyric acid (GABA) agonist, muscimol, but differs fundamentally from that associated with ether-induced coma or that induced by infusing ammonia or a mixture of ammonia, a mercaptan precursor and a fatty acid. As barbiturates, benzodiazepines and GABA agonists are known to mediate their neuroinhibitory effects by interacting with post-synaptic receptors for γ-aminobutyric acid (GABA) in the central nervous system, these observations suggest that activation of the GABA inhibitory neurotransmitter system may contribute to the neural inhibition of HE.

GABA metabolism and the status of the GABA neurotransmitter system have been investigated in HE. It has been shown that (i) a major source of GABA in peripheral blood is the enteric bacterial flora; (ii) plasma levels of GABA-like activity are increased by more than an order of magnitude in HE but not in another type of metabolic encephalopathy; (iii) as the liver fails plasma levels of GABA become elevated before the onset of HE; and (iv) the density of receptors for GABA on postsynaptic neural membranes of the brain is increased in acute liver failure. To determine whether GABA in plasma can gain access to the brain, the transport of the non-metabolizable isomer of GABA, α-aminoisobutyric acid (AIB), across the blood brain barrier was measured by a computer-assisted quantitative autoradiographic technique. Blood to brain transport of ^{14}C-AIB is minimal in normal rabbits but with the onset of liver failure it increases markedly in specific gray matter areas of the brain prior to the onset of HE. These findings suggest that as the liver fails plasma GABA derived from the gut crosses an abnormally permeable blood-brain barrier before the onset of HE and contributes to the neural inhibition of HE. The increase in the number of GABA receptors in HE may be associated with enhanced sensitivity of the brain to GABA-ergic neural inhibition.

The number of binding sites for benzodiazepines on postsynaptic membranes is also increased in HE, suggesting that the benzodiazepine binding site and the GABA receptor are regulated as a single operational unit and that the increased sensitivity to benzodiazepines in HE is due to the presence of more benzodiazepine receptors permitting increased drug effect.

Clearance of ^{3}H-GABA from plasma is markedly decreased in liver failure, primarily due to inefficient hepatic extraction. Studies with isolated hepatocytes indicate that GABA binds specifically to the high affinity acceptor of the A amino acid transport system. Thus, impaired function of this transport system in liver failure could contribute to increased plasma GABA levels.

Studies of the binding of GABA to post-synaptic membranes prepared from brains obtained at human autopsies and measurements of plasma GABA levels in patients with a variety of liver and other diseases are being conducted to determine whether the findings obtained with the rabbit model of FHF apply to man. Preliminary results indicate that the number of GABA receptros is increased in the brains of patients dying with acute or chronic hepatocellular failure but not in the brains of patients dying from a variety of other causes. Furthermore, plasma levels of GABA-like activity were found to be elevated about fourfold in patients with decompensated cirrhosis without overt HE but were elevated more than tenfold in patients with cirrhosis complicated by HE or a gastrointestinal hemorrhage or with fulminant hepatic failure.

Hyperammonemic encephalopathy differs fundamentally from HE in terms of the associated changes in visual evoked potentials and receptors for the excitatory neurotransmitter glutamate. In HE the number of glutamate receptors decrease without change in affinity whereas in hyperammonemia both the number and affinity of these receptors increase. These findings suggest that hyperammonimia is not a good model of HE. Further studies of the status of neurotransmitter receptors have indicated that in HE there is a selective increase in receptors for inhibitory amino acid neurotransmitters, a selective decrease in receptors for excitatory amino acid neurotransmitters and no change in the number of receptors for acetylcholine, opiates, enkaphalins and dopamine. These findings are ompatible with increased sensitivity to inhibitory neurotransmitters and decreased sensitivity to excitatory neurotransmitters contributing to the neural inhibition of HE.

HE is associated with an increase in the lipid:protein ratio of synaptic plasma membranes without associated changes in membrane fluidity or major membrane proteins. The altered composition of synaptic plasma membranes in HE may be the basis of changes in neurotransmission in this syndrome. [E. A. Jones, K. D. Mullen, M. Bassett, J. G. Waggoner, P. Munson (NICHHD)].

II. Studies of Cellular Immune Function in Primary Biliary Cirrhosis

The role of abnormal immune mechanisms in the pathogenesis and perpetuation of the hepatobiliary lesion of primary biliary cirrhosis (PBC) is being studied. Recent studies have been designed to determine whether there is a defect in immune regulation in patients with PBC. T lymphocyte-mediated help and suppression of pokeweed mitogen-induced immunoglobulin (Ig) synthesis by cultured B lymphocytes have been quantitated in vitro using sensitive radioimmunoassays to measure IgG and IgM. The results suggest that there is deficient suppression of Ig synthesis in PBC due to a T cell defect.

The suppressor T cell defect in PBC is not secondary to chronic cholestasis or hepatocellular disease per se and the defect exhibits specificity for PBC amon liver diseases.

It has been shown that T cells can be induced to proliferate when cultured with either autologous or allogeneic irradiated B cells. These phenomena are known as the autologous and allogeneic mixed lymphcoyte reactions (MLR), respectively. In patients with PBC, the mean autologous, but not allogeneic, MLR was found to be significantly less than that for controls. Thus, deficiencies of both suppressor T cell function and the autologous MLR are found in PBC. These deficiencies could be due to a common defect of cell interactions involved in the generation of suppressor effector cells. Such a defect could play an important role in the pathogenesis of PBC by providing an abnormal immunologic environment which predisposes to the development of autoimmune phenomena and a state of immune hyperresponsiveness.

Cytotoxicity mediated by circulating natural killer (NK) cells is reduced in PBC. This phenomenon is not due to a reduced number of effector cells, inhibitory serum factors or suppressor cells. The defect can be partially corrected by incubating PBC NK cells with interferon.

Complement receptor function in PBC has been assessed by quantitating the ability of peripheral blood monocytes to form rosettes with complement-coated sheep erythrocytes. PBC monocytes have a normal capacity to form rosettes but PBC serum in the presence or absence of normal serum inhibits rosette formation. This inhibition is mediated by an IgM-containing fraction of PBC serum and does not depend on complement. Thus, the previously demonstrated defective complement receptor-mediated clearance function in PBC appears to be due to t presence of serum factor which blocks complement receptors on macrophages. [E. A. Jones, S. P. James (NIAID), M. I. Avigan, and W. Strober (NIAID)].

III. Studies of Protease Inhibitor Phenotypes

Pi phenotypes and serum α-1-antitrypsin (α1AT) concentrations have been determined in 80 unselected southern African Black patients with hepatocellular carcinoma and 103 age, sex and tribally matched control subjects. Non-MM phenotypes were present in 8.7% of patients with hepatocellular carcinoma and 12.6% of controls. The heterozygous PiZ carrier state was present in 5.0% of patients with hepatocellular carcinoma and 1.9% of controls; no subject had the homozygous PiZZ phenotype. No patient with hepatocellular carcinoma had a subnormal serum α1AT concentration. It is inferred that α1AT deficiency does not play an etiologic role in hepatocellular carcinoma in southern African Blacks. [E. A. Jones and J. Vergalla].

IV. Studies of Chronic Hepatitis B Surface Antigen (HBsAg) Carriers

Over one hundred HBsAg-positive patients with chronic liver disease of varying degrees of severity have been enrolled in this project. Spontaneous complete resolution of chronic type B hepatitis, with loss of serum HBsAg, occurs in about 1% of patients per year but spontaneous remissions in disease activity occur in about 14% of patients each year. These remissions are characterized by disappearance of serum HBV-DNA and DNA polymerase activity, seroconversion from HBeAg to anti-HBe and a fall in elevated serum aminotransferase levels to values within the normal range.

A randomized controlled trial of a 28 day course of the anti-viral drug adenine arabinoside 5'-monophosphate (Ara-AMP) has been conducted in 20 patients with HBeAg positive chronic type B hepatitis. Ara-AMP led to a dramatic and usually complete inhibition of serum levels of DNA polymerase and HBV-DNA. However, when therapy was stopped, the levels of these viral markers returned to pretreatment values in most patients. Two of 10 treated patients and 2 of 10 non-treated patients have exhibited a long lasting remission of disease activity (as defined above). The most troublesome side effect of Ara-AMP was severe muscle pains lasting up to 7 weeks after stopping the drug.

To determine if withdrawal of immunosuppressive therapy is associated with a higher incidence of remissions in disease activity than occurs spontaneously, a double-blind controlled trial of a one-month high dose course of prednisolone for patients with chronic type B hepatitis has been conducted. None of 15 patients entered into this trial has undergone a remission of disease activity. Trials of therapy with recombinant DNA human leukocyte alpha interferon for patients with chronic type B hepatitis have been initiated. [J. H. Hoofnagle, M. Peters, M. Avigan, S. Straus (NIAID), R. Johnson (NIAID), R. H. Purcell (NIAID), G. L. Davis, J. S. Dooley and E. A. Jones].

V. Controlled Trial of Chlorambucil Therapy in Primary Biliary Cirrhosis

Primary biliary cirrhosis (PBC) is considered to be an autoimmune disease. It is characterized by slowly progressive intrahepatic cholestasis due to a chronic non-suppurative destructive cholangitis involving the septal and larger interlobular bile ducts. When symptomatic the disease appears to be uniformly fatal. So far trials of immunosuppressive drugs in this disease have not effectively inhibited the progression of the disease or increased survival. Because certain other chronic inflammatory and presumably autoimmune diseases appear to respond favorably to alkylating agents but not to azathioprine, a controlled randomized trial of the alkylating agent chlorambucil has been initiated in patients with PBC. Twenty-four patients have been admitted to the trial; 13 have been randomized to the treatment group and 11 to the control group. The dose is titrated so that a polymorphonuclear leucocyte count of at least 1000/mm^3 is maintained while the lymphocyte count is decreased to about 50% of pretreatment values. Two patient have died in the control group. Complications of chlorambucil therapy led to discontinuing the drug in two patients. Follow-up liver biosies have revealed a decrease in hepatic inflammatory activity in 9 of 11 treated patients, but in only 3 of 11 untreated patients. [E. A. Jones, J. H. Hoofnagle, M. Peters, M. I. Avigan, S. C. Pappas, G. L. Davis, K. D. Mullen].

VI. Studies of Chronic Non-A, Non-B Hepatitis

Patients with well-defined chronic non-A, non-B hepatitis are being evaluated. The natural history of this condition is being studied in collaboration with Dr. Harvey J. Alter (NIH Clinical Center Blood Bank). Twenty-eight patients with HBsAg negative post-transfusion hepatitis whose serum levels of aminotransferases remained elevated for more than six months have been evaluated and have undergone percutaneous liver biopsy. These pateints have had evidence of hepatitis for periods varying between 2 and 11 years. Seven experience mild degrees of lethargy or malaise; the remainder have no symptom attributable to liver disease. Serum aminotransferase levels fluctuate from normal values to as high as 1500 IU/l. The disease is not associated with an increased incidence of any HLA types. Liver biopsies have revealed chronic active hepatitis in 21 patients, chronic persistent hepatitis in 7 and alcoholic liver disease in 2. Six patients have compensated cirrhosis. Follow-up liver biopsies from 13 patients have demonstrated progession of the liver disease in 4, and milder degrees of chronic hepatocellular disease in the rest.

In a pilot study in which the antiviral drug Acyclovir was administered IV three times a day for 2 weeks to 5 patients with chronic non-A, non-B hepatitis, no consistent effect of the drug on elevated serum aminotransferase levels was observed. [J. H. Hoofnagle, H. J. Alter (CC:BB), E. A. Jones, R. H. Purcell (NIAID), G. L. Davis, S. M. Feinstone (NIADDK), K. Ishak (A.F.I.P.), M. Peters, and M. I. Avigan, K. D. Mullen].

VII. Immunological Studies in Chronic Hepatocellular Disease

The role of immunologic mechanisms in the perpetuation of chronic viral hepatitis is being studied and the effects of different therapies for chronic viral hepatitis on the immune system are being evaluated. The role of interferon in the etiology and treatment of chronic viral hepatitis is also being assessed. Alpha interferon production by lymphocytes of patients with untreated chronic type B hepatitis is decreased and when such patients are treated with high doses of interferon, alpha interferon production by lymphocytes is decreased further. When a subgroup of patients with chronic t B hepatitis, whose lymphocytes synthesize appreciable quantities of immunoglobulins and are particularly responsive in proliferation assays, are treated with interferon serum markers of hepatitis B viral replication disappear. Interferon therapy in responders was associated with a decrease in immunoglobulin production by lymphocytes. Receptors for interferon on peripheral blood mononuclear cells are being characterized. [J. H. Hoofnagle, J. S. Dooley, M. Peters, M. I. Avigan, E. A. Jones].

PHOENIX CLINICAL RESEARCH SECTION

Research at the Phoenix Clinical Research Section is in three major areas: 1) non-insulin dependent diabetes mellitus 2) obesity and energy balance, and 3) lipoprotein metabolism. Investigations in these areas have been both in vivo and in vitro.

Diabetes mellitus

The Indians of the Gila River Indian reservation have the highest reported prevalence and incidence rates of non-insulin dependent diabetes mellitus (NIDDM) of any population in the world. Obesity is a major risk factor for the development of NIDDM. Diabetes also occurs much more frequently in the offspring of a diabetic mother than in offspring of non-diabetic parents. The reasons for this are unknown. The major effort of our research section in the past two years has been a cross-sectional and longitudinal study of the offspring of the two parental types. We have been studying many aspects of carbohydrate metabolism in vivo and in vitro in these subjects. Based on previously collected data from the Epidemiology and Field Studies Branch in Phoenix we know that approximately 30% of the offspring of diabetic mothers will develop NIDDM within 5 years. This will make it possible to determine which metabolic parameter(s) studied, if any, is (are) predictive of NIDDM.

To date about 150 subjects have been entered into the cross-sectional phase of the study. We have also re-admitted over 50 subjects for their first year follow-up. During their admission each subject undergoes a thorough medical history and physical examination with anthropomorphic measurements. Over the next two weeks each subject has an oral glucose tolerance test, an underwater weighing for body composition determination, intravenous glucose tolerance test, measurement of insulin and C-peptide responses to a 24 hour standard diet of mixed composition, hyperinsulinemic euglycemic clamp with simultaneous indirect calorimetry at a low and maximal insulin concentrations, an open abdominal fat biopsy and a maximal oxygen uptake determination.

Ongoing analyses are performed on this rapidly growing large data base. Some important and interesting observations have already been made. Increasing obesity is only weakly associated with evidence of a "post-receptor defect" in insulin action in vivo and in vitro in isolated abdominal adipocytes. Surprisingly, there were only very weak correlations between maximal insulin stimulated glucose transport in isolated adipocytes and the maximal insulin stimulated glucose disposal rate in vivo. Maximal oxygen uptake however was significantly correlated with maximal insulin action at the high insulin concentrations. At lower insulin concentrations, obesity appeared to be better correlated with insulin action over a range of percent body fat up to approximately 25-30%. Above this degree of obesity there was little or no correlation between adiposity and in vivo insulin action. The maximal oxygen uptake was well correlated with insulin action over the entire range of uptakes. The reasons for the good correlation between the maximal oxygen uptake and insulin action in vivo, both at submaximal and maximal insulin concentrations, is unexplained and will require further study.

Indirect calorimetric data has enabled us to also assess the relationships between degree of obesity and maximal oxygen uptake and insulin mediated glucose oxidation and glucose disposal by non-oxidative pathways, or "glucose storage". The data suggest that differences in net glucose disposal between very insulin sensitive and extremely insulin resistant subjects are mainly due to the differences in non-oxidative disposal rates.

Since the degree of obesity was correlated with insulin action over only part of the range of adiposity, and only at low plasma insulin concentrations, other pathophysiologic mechanisms for the determination of reduced in vivo insulin action have been sought. One hypothesis to explain the insulin resistance observed in obese subjects is that it is a result of overnutrition rather than the increased fat mass per se. We studied 15 male Indians before and after 10 days of a 60% increse in caloric intake. The increased caloric intake was associated with reduced in vivo insulin action, particularly for non-oxidative glucose disposal. There also was an associated reduction in muscle glycogen synthase activation by insulin, and paradoxically increased maximal insulin stimulated glucose transport rate into adipocytes. The results suggested that the reduced insulin action observed in obese subjects may be in part a result of overnutrition rather than the increased fat mass per se.

Alternatively, the increased fat mass could affect glucose disposal in man through the so called "Randle effect". We have evaluated this hypothesis by examining the relationship between free fatty acid turnover, using labelled palmitate, free fatty acid concentrations and insulin mediated glucose, glucose oxidation, and storage rates. The results of this study suggested that the Randle effect is operative in man. That is, reduced glucose oxidation rates were observed in subjects with increased lipid oxidation rates, and lipid oxidation increased in direct proportion to the free fatty acid concentration. There was however no correlation between the free fatty acid concentration or turnover and nonoxidative glucose disposal or glucose storage rates. Since glucose storage rates were better correlated with total glucose disposal, there was little correlation between free fatty acid concentrations and insulin action. Thus we concluded that in obese subjects the increased fat mass effects changes in insulin mediated glucose disposal only in the oxidative pathway. Since the oxidative pathway does not account for most of the difference in glucose disposal between sensitive and resistant subjects, this effect is only a small part of the pathophysiologic mechanism that must determine insulin resistance in obese subjects.

Since glucose storage rates seem to be the major difference between sensitive and insulin resistant subjects we also determined the effect of muscle glycogen depletion on in vivo insulin action in man. The results suggested that muscle glycogen depletion is associated with increased in vivo insulin action in man, and that the increased insulin action was proportional to the increase in muscle glycogen synthase activity.

These studies have increased our understanding of the determinants of in vivo insulin action in man. However, previous studies have left unclear as to what extent reduced insulin action plays a role in the pathogenesis in non-insulin dependent diabetes mellitus. The confusing data in this regard may be largely a result of the fact that no study is available in which both measures of insulin action and insulin secretion were made in the same group of subjects. We therefore have measured these parameters in a group of 34 Indian volunteers. The results show that reduced insulin action is likely to be resposible for changes in fasting glycemia up to concentrations of approximately 120 ml/dl. Above that concentration there is little further reduction in insulin action, but further reductions in peripheral insulin concentrations. The increase in fasting glycemia is also directly proportional to the endogenous glucose production rate and the fasting free fatty acid concentration. We hypothesize that the increase in fasting glycemia above approximately 120 mg/dl is a result of falling peripheral plasma insulin concentrations which result in increased free fatty acid concentrations and an increased flux of gluconeogenic substrates to the liver. The increased free fatty acid concentrations and substrates stimulate gluconeogenesis and increase hepatic glucose production resulting in progressive hyperglycemia. We will be further testing this hypothesis in a weight-reduction protocol designed to determine the relationship between changes in fasting glycemia, hepatic glucose production, and changes in gluconeogenic substrates supplied to the liver.

We have also pursued the mechanisms of reduced insulin action at the cellular level in vitro. Firstly, since reduced glucose storage rates appeared to be largely response for the differences between insulin sensitive and insulin resistant subjects, and also for the decreased glucose disposal in the diabetics, we examined the relationship between glucose storage rates and muscle glycogen synthase activity under a variety of conditions. The glucose storage rate and muscle glycogen synthase activity are well correlated under resting conditions, after muscle glycogen depletion, and with the reduction in insulin action accompanying overnutrition there is also a reduction in muscle glycogen synthase activity. Whether this is a cause and effect relationship is unknown, however, we will be continuing to test this hypothesis in subsequent experiments. In addition we have continued efforts to assess the determinants of in vivo insulin action in vitro in isolated abdominal adipocytes.

Results to date have shown that adipocytes from diabetic subjects have altered adipocyte metabolism characterized by decreased insulin sensitivity of glucose transport, decreased maximal insulin stimulated glucose transport and metabolism as well as increased basal lipolysis but without any change in insulin binding. We have also shown that the capacity defects, but not the sensitivity defects, can be improved with insulin therapy. Recently we have also found that the sensitivity of glucose transport to insulin (i.e. 1/ED 50) in subjects with abnormal glucose tolerance were about half of that found in subjects with normal glucose tolerance. This was in spite of the fact that the subjects were matched for age, sex, obesity, fasting plasma glucose and insulin concentrations in adipose cell size. There were also no differences in the capacity of transport, metabolism, lipolysis, insulin binding, or the sensitivity of ntilipolysis to insulin. Thus the sensitivity to glucose transport to insulin appeared to be lower in subjects with abnormal glucose tolerance due to a post binding defect in the coupling of insulin binding to glucose transport. This defect preceded the increased glycemia associated with NIDDM. This may explain why this sensitivity defect, which is also present in diabetics, was not improved with insulin therapy while the capacity parameters were. The reduction in ED 50 for insulin stimulated glucose transport were correlated with in vivo insulin action. The results paralleled the relationships between in vivo insulin action and muscle glycogen synthase activation.

We are also now investigating in more depth the control of glucose transport in insulin sensitive tissues. We have previously shown that 2 deoxy glucose transport is lower than 3-0-methyl-glucose transport and that preincubation with 10 mm glucose can accelerate the 2-deoxy-glucose transport rate up to the 3-0-methyl-glucose transport rate. We are now investigating the amount of time that glucose actually has to be present during this incubation period to obtain the 2-deoxy-glucose acceleration. In the likely event that this period is shorter than 5 minutes we will investigate whether the effect is protein synthesis dependent.

Because of the discordant results observed in adipocytes in vitro and in vivo insulin action in man, and because of good correlations between glucose storage rates with in vivo insulin action and the absence of a good relationship between glucose oxidation and in vivo insulin action, many questions have been raised concerning the true rate limiting steps for glucose uptake in glucose tolerant and diabetic man. In order to investigate the possibility that glucose uptake into muscle is not rate limited by glucose transport under all conditions, we are setting up the rat hind-limb perfusion technique and will measure glucose uptake into muscle over a range of glucose and insulin concentrations. We will eventually look at which metabolic steps beyond transport, for example oxidation or glycogen synthesis that might be rate limiting under given glucose and insulin concentrations.

Obesity

It has been proposed that the high prevalence of obesity amongst the Gila River Indians may be due to a genetic selection for a "thrifty gene". In order to determine if such genetic differences exist, resting metabolic rates and responses to various thermogenic stimuli including food, norepinephrine and exercise have been measured in these Indians during weight maintenance and during overfeeding. These studies were done in lean and obese individuals. It appears that obese Pima Indians, though having similar basal norepinephrine concentrations achieve much higher concentrations of the hormone during identical norepinephrine infusions (based on fat free mass). These data, coupled with analysis of norepinephrine decay curves have suggested the presence of a norepinephrine clearance defect in obese Indian subjects.

We have also examined the thermogenic response of Indian subjects to insulin and glucose infusions. Preliminary data suggests that there is a markedly reduced increase in energy expenditure in Indians, compared to Caucasians, in response to insulin and glucose infusions. This was most apparent at low plasma insulin concentrations. At very high or maximally stimulating insulin concentrations the thermogenic response of the Indians was quite comparable to that of Caucasians. These preliminary data suggest the possible presence of a thermogenic defect in the Indian subjects. The significance of these differences in thermogenesis in regards to the higher prevalence of obesity in this population remains to be determined however.

Obesity is much more prevalent in the children who are the product of a diabetic pregnancy than in children of a normal pregnancy. We have now begun a study to further characterize the body composition of these two groups of children. Body composition is determined by heavy water dilution and by underwater weighing. Percutaneous needle biopsies are also done to determine the fat cell size in the abdominal and gluteal adipose tissue depots.

Lipoproteins

Both low density lipoproteins and very low density lipoproteins have been implicated as positive risk factors for coronary vascular disease. High density lipoprotein concentration is an independent negative risk factor. Both diabetes and obesity have been associated with changes in plasma lipoproteins and these changes may be related to the increased prevalence of cardiovascular disease in some populations. We are studying the lipoprotein composition of obese and diabetic Pima Indians as compared to Caucasians. Pima males and females have lower total and LDL cholesterol. Pima males and females have lower HDL cholesterols than Caucasians and there is no sex difference in HDL in the Pimas. Measurement of total cholesterol and triglyceride concentrations on two South Pacific populations revealed similar data to the Pimas. The data suggest that there are racial differences in lipoprotein distributions which may be important in the determination of the prevalence of coronary vascular disease in various racial groups.

The mechanisms by which reduced low density lipoprotein synthesis and low plasma cholesterol levels are maintained in the Pima Indians is unknown. This is an active area of investigation by our laboratory in order to understand the control of lipoprotein metabolism and how lipoprotein metabolism is influenced by obesity and diabetes. Simultaneous studies have been performed of very low density lipoprotein apoB and LDL apoB metabolism using a double isotope procedure. Studies in male Pima Indians indicated that obesity results in greatly increased flux of VLDL apoB. LDL apoB levels were not elevated in the obese subjects because of both increased metabolism of VLDL through the "alternate pathway" and increased catabolism of LDL. Comparison of apoB metabolism in Pimas and weight matched Caucasians indicated that the low LDL in Pimas is related to both higher fractional catabolic rates for LDL and higher proportion of VLDL metabolized without conversion to LDL. Furthermore, this VLDL "shunt" and LDL fractional catabolic rate appear to be correlated. These data

~uggest a large flux of substrates and regulators which cause overproduction of lipoproteins but that compensatory mechanisms are operative resulting in maintenance of low plasma concentrations.

Also recent studies in non-insulin dependent diabetics in this population have shown an elevation of plasma free fatty acids in proportion to the fasting blood sugar. The increased free fatty acid concentration appeared to be both a result of increased production and impaired removal of free fatty acids. The impaired removal of free fatty acids was closely related to glycemic control suggesting there may be impaired reesterification of free fatty acids in poorly controlled diabetes mellitus. Treatmetn with sulfonylurea therapy resulted in improvement in glycemic control and was associated with significant reductions in VLDL triglyceride and LDL cholesterol but no changes in HDL and HDL subfractions. Treatment appeared to have independent effects on the metabolism of VLDL triglyceride and VLDL apoB since in the former the transport was decreased after treatment whereas for the latter transport was unchanged and fractional catabolc rate was increased.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 53001-14 DDB

PERIOD COVERED
October 1, 1983 to Sept.30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of Membrane Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jerry D. Gardner	Chief	DDB, NIADDK
Others:	R. T. Jensen	Senior Investigator	DDB, NIADDK
	M. J. Collen, K. E. McArthur	Clinical Associates	DDB, NIADDK
	J. Howard, M. Noguchi, P. Maton	Visiting Scientists	DDB, NIADDK
	M. Spanarkel, J. Cherner, S. Wank	Clinical Associates	DDB, NIADDK
	T. Garvey, G. Delle Fave	Guest Workers	DDB, NIADDK
	D. Kasbekar	Guest Worker	DDB, NIADDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Digestive Diseases Branch

SECTION
Section on Gastroenterology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	3.6	1.6

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The broad categories which are included in the project are: 1)Characterizing functionally the mechanism by which various substrates cross the plasma membrane of different mammalian cells; 2) identifying the metabolic and humoral factors which influence the transport of various substrates across the plasma membrane; 3) developing techniques which will distinguish between binding of a substrate to the membrane and translocation of the substrate across the membrane; 4) characterizing the mechanism by which the membrane transport of various substrates is altered in certain diseases; and 5) relating these alterations of membrane transport to the pathogenesis and clinical manifestations of the disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 53002-12 DDB

PERIOD COVERED
October 1, 1983 to Sept. 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Gastrointestinal Hormones

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. D. Gardner	Chief	DDB, NIADDK
Others:	R. T. Jensen	Senior Investigator	DDB, NIADDK
	K. E. McArthur, M. Spanarkel	Clinical Associates	DDB, NIADDK
	B. M. Bissonnette	Clinical Associate	DDB, NIADDK
	J. Howard, M. Noguchi, P. Maton	Visiting Scientists	DDB, NIADDK
	J. Cherner, S. Wank	Clinical Associates	DDB, NIADDK
	G. Delle Fave, D. Kasbekar	Guest Workers	DDB, NIADDK

COOPERATING UNITS (if any)
Dept. of Chemistry, Case-Western Reserve Univ., Cleveland, Ohio
Div. of Cellular Biology, Kennedy Institute for Rheumatology, London, England

LAB/BRANCH
Digestive Diseases Branch

SECTION
Section on Gastroenterology

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.6	4.0	1.6

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In vitro systems are being used to study the mechanism of action of gastrin, secretin, cholecystokinin, bombesin, substance P and vasoactive intestinal peptide with their specific membrane receptors.

Clinical investigators are directed toward developing alternative forms of therapy for and elucidating the pathogenesis of disorders characterized by ectopic production of gastrointestinal hormones (e.g., Zollinger-Ellison syndrome and pancreatic cholera).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 53004-12 DDB

PERIOD COVERED
October 1, 1983 to Sept. 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cyclic Nucleotide Mediated Functions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jerry D. Gardner	Chief	DDB, NIADDK
Others:	R. T. Jensen	Senior Investigator	DDB, NIADDK
	M. J. Collen, K. E. McArthur	Clinical Associates	DDB, NIADDK
	M. Spanarkel, B. M. Bissonnette	Clinical Associates	DDB, NIADDK
	J. Howard, M. Noguchi, P. Maton	Visiting Scientists	DDB, NIADDK
	S. Wank, J. Cherner	Clinical Associates	DDB, NIADDK
	G. Delle Fave, D. Kasbekar	Guest Workers	DDB, NIADDK
	T. Q. Garvey, M. Jackson	Guest Workers	DDB, NIADDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Digestive Diseases Branch

SECTION

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS: 6.6	PROFESSIONAL: 5.0	OTHER: 1.6

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In vitro systems are being used to characterize the mechanism by which cyclic nucleotides alter cell function and to explore the mechanism of action of agents whose effect on cell function is mediated by cellular accumulation of cyclic nucleotides.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 53,500-11 DDB

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of the Transport of Organic Anions by the Liver

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

E. Anthony Jones, M.D. Chief, Liver Diseases Section A:DDB

COOPERATING UNITS *(if any)*

LAB/BRANCH

Digestive Diseases Branch

SECTION

Liver Diseases Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: .25	PROFESSIONAL: .25	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is currently inactive.

PHS 6040 (Rev. 1/83) GPO 895-100

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AM 53,501-11 DDB

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of the Pathogenesis of Acute Hepatic Coma

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
E. Anthony Jones, M.D. Chief, Liver Diseases Section A:DDB

COOPERATING UNITS *(if any)*
RR:NICHHD
CP:NCI

LAB/BRANCH
Digestive Diseases Branch, NIADDK

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1.5	.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The abnormal pattern of visual evoked potentials in rabbits with hepatic encephalopathy (HE) due to fulminant hepatic failure (FHF) resembles that associated with coma induced by a barbiturate, a benzodiazepine or a -aminobutyric acid (GABA) agonist. As these drugs induce neural inhibition by interacting with binding sites on the GABA receptor complex on postsynaptic neural membranes, these findings suggest that neuronal activity in HE may be similar to the neuronal inhibition induced by GABA. Outside the CNS the main source GABA is gut bacteria and the main site of its catabolism is the liver. When FHF was induced in rabbits the onset of HE was preceded by a marked increase in the plasma levels of GABA-like activity and by a nonspecific increase in the permeability of the blood brain barrier (BBB). FHF was associated with significant increases in the densities of brain receptors for the inhibitory amino acid neurotransmitters, GABA and glycine, and for benzodiazepines. FHF was also associated with significant decreases in the densities of brain receptors for the excitatory amino acid neurotransmitters, glutamate and aspartate. FHF was not associated with any change in the densities of brain receptors for four non-amino acid neurotransmitters. In FHF there is an increase in the content of cholesterol and phospholipid relative to that of protein in brain synaptic membranes. These findings suggest that as the liver fails: 1) impaired hepatic metabolism of GABA leads to an increase of gut-derived GABA in plasma and 2) plasma GABA gains access to the brain through a permeable BBB and induces neural inhibition. Changes in the composition of neural membranes and neurotransmitter receptors in liver failure may render the brain less sensitive to excitatory amino acid neurotransmitters and more sensitive to inhibitory amino acid neurotransmitters.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 53,503-10 DDB

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunologic Studies of Primary Biliary Cirrhosis

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

E. Anthony Jones, M.D. Chief, Liver Diseases Section A:DDB

COOPERATING UNITS *(if any)*

Mucosal Immunity Section, Laboratory of Clinical Investigation, NIAID

LAB/BRANCH

Digestive Diseases Branch

SECTION

Liver Diseases Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	.5	.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Abnormal immune mechanisms are being studied in patients with primary biliary cirrhosis (PBC). T cell mediated help and suppression of pokeweed mitogen-induced immunoglobulin synthesis by B cells has been studied using radioimmunoassays to measure IgG and IgM synthesized by cultures containing appropriate mixtures of different lymphocyte subpopulations in vitro. The ability of T cells to proliferate when cultured with either autologous or allogeneic irradiated B cells (mixed lymphocyte reactions) or with hapten-modified autologous irradiated B cells has been assessed. Results of these studies include the demonstration in PBC of (i) a diminished capacity of T cells to inhibit immunoglobuin synthesis in vitro; (ii) a deficiency of the autologous but not the allogeneic mixed lymphocyte reaction and (iii) a deficiency in the primary but not the secondary proliferative response of T cells to hapten-modified cells. These findings suggest that in PBC there is a fundamental defect in the interaction between autoreactive T cells and surface antigens on autologous non-T cells which leads to diminshed activation of suppressor T cells and hence predisposes to a state of immune hyperresponsiveness.

PHS 6040 (Rev. 1/83) GPO 895-100

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AM 53,505-09 DDB

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Alpha-1-Antitrypsin Phenotypes and Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

E. Anthony Jones, M.D. Chief, Liver Diseases Section A:DDB

COOPERATING UNITS *(if any)*

Pulmonary Branch, NHLBI

LAB/BRANCH

Digestive Diseases Branch

SECTION

Liver Diseases Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	.25	.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Isolated preparations of α-1-antitrypsin (α1AT), trace-labeled with radioiodine, have been used to obtain data on the turnover of α1AT in normal subjects and patients with α1AT deficiency who have either pulmonary emphysema or chronic hepatocellular disease. The protease inhibitor (Pi) phenotypes of subjects donating plasma for isolation of α1AT and subjects undergoing α1AT turnover studies have been determined. The fractional catabolic rates of M α1AT from normal subjects (phenotype PiMM) and Z α1AT from patients with α1AT deficiency (phenotype PiZZ) are similar in both normal subjects and patients with α1AT deficiency. These findings imply that the low serum α1AT concentration in α1AT deficiency is due to impaired hepatic release of α1AT and that the differences in molecular structure between the Z and M proteins have little effect on the catabolism of α1AT. In common with other glycoproteins, desialylated (i.e. neuraminidase-treated) α1AT is rapidly cleared from plasma by the liver. Pi phenotypes have been determined in populations of normal subjects and patients with rheumatoid arthritis, systemic lupus erythematosus, Sjogren's syndrome and hepatocellular carcinoma.

PHS 6040 (Rev. 1/83) GPO 895-100

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 53,508-07 DDB

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Hepatic Receptors for Glycoproteins

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

E. Anthony Jones, M.D. Chief, Liver Diseases Section A:DDB

COOPERATING UNITS *(if any)*

Labortory of Biochemistry and Metabolism

LAB/BRANCH

Digestive Diseases Branch

SECTION

Liver Diseases Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.5	.25	.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The cellular location and carbohydrate specificies of a glycoprotein recognition system on rat hepatic sinusoidal cells have been determined. Purified preparations of endothelial, Kupffer and parenchymal cells have been prepared in situ by collagenase liver perfusion, centrifugation on Percoll gradients and centrifugal elutriation. ^{125}I-labeled agalactoorosomucoid (AGOR), an N-acetylglucosamine-terminated glycoprotein, is selectively and specifically taken up in vitro by endothelial cells. Glucose and a glucose-albumin conjugate competitively inhibited this uptake process over a wide range of concentrations. Uptake by cells from fasted rats was enhanced, but uptake of cells from fasted or fed diabetic rats was normal. The in vivo hepatic uptake and catabolism of ^{125}I-AGOR were slower in diabetic than normal rats. These fidings indicate that 1) the hepatic receptors which recognize N-acetylglucosamine/mannose terminated glycoproteins are located on endothelial cells, 2) these receptors are glucose sensitive, 3) fasting increases the number of these receptors and 4) diabetes mellitus abolishes this effect of fasting and impairs the function of this receptor in vivo. The results of this study suggest a mechanism for abnormal glycoprotein metabolism in diabetes mellitus.

PHS 6040 (Rev. 1/83)

GPO 895-100

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AM 53,509-06 DDB

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of patients who are chronic carriers of hepatitis B surface antigen

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
Jay H. Hoofnagle, M.D. Senior Investigator A:DDB

COOPERATING UNITS *(if any)*
LID and LCI, NIAID
Georgetown University School of Medicine; VA Hospital, Washington, DC
Viral Diseases Section, Walter Reed Army Institute of Research, Washington, D.C.

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A cohort of patients with chronic type B hepatitis has been followed regularly with frequent determinations of routine serum biochemical liver tests as well as serological markers of hepatitis B virus infection. Selected individuals have been entered into therapeutic trials of antiviral and immunomodulating therapies. Three forms of therapy have been investigated: 1) adenine arabinoside monophosphate (Ara-AMP), 2) corticosteroids and 3) recombinant DNA human leukocyte alpha interferon (IFN). A total of 26 patients were treated with Ara-AMP in various dosage regimens over the past 3 years. Long-term follow-up has demonstrted that only 2 of the 26 (8%) have had a sustained beneficial clinical remission in response to therapy. This rate of response is similar to the spontaneous rate of remission in untreated controls. A short course of high dose prednisolone therapy was given to 10 patients in a randomized, double blind controlled trial. During a one-year follow-up, none of the 10 predniosolone-treated and none of five placebo-treated patients underwent a remission in the disease. Indeed, in three treated patients the disease activity increased. In a pilot study, eight patients were treated with multiple courses of a highly purified recombinant human leukocyte interferon. Subsequently, a sustained clinical and serological remission occurred in two of the eight patients. A randomized controlled trial of a four-month course of recombinant human leukocyte interferon has been begun.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 53,510-05 DDB

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of the Natural History and Treatment of Chronic Non-A, Non-B Hepatitis

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

E. Anthony Jones, M.D. Chief, Liver Diseases Section A:DDB

COOPERATING UNITS *(if any)*

NIH Blood Bank
Hepatitis Section, LID:NIAID
Armed Forces Institute of Pathology

LAB/BRANCH

Digestive Diseases Branch

SECTION

Liver Diseases Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.75	.75	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients with well-documented post-transfusion chronic non-A, non-B (non-lupoid) hepatitis are being evaluated to determine the natural history of this disease and to obtain tissues and reagents to search for non-A, non-B agents. Thirty patients have been admitted to this study. These patients have undergone thorough evaluations including a liver biopsy. They have had the disease for periods ranging from 1 to 11 years. Seven patients have a mild degree of malaise, the remaining patients are asymptomatic. Liver biopsies have revealed chronic persistent hepatitis in seven, chronic active hepatitis in twenty-one and alcoholic liver disease in two. Six patients have developed cirrhosis and one of these has died of hepatocellular failure. Trials of immunosuppressive and antiviral therapy for these patients are being planned. A pilot study of Acyclovir therapy has been carried out in five patients. No consistent effect of the drug on aminotransferase levels was observed. A pilot study of recombinant human leukocyte interferon has been initiated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AM 53,511-05 DDB

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Controlled Trial of Chlorambucil in Primary Biliary Cirrhosis

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
E. Anthony Jones, M.D. Chief, Liver Diseases Section A:DDB

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1.5	0.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Primary biliary cirrhosis (PBC) is a disease of unknown etiology characterized by slowly progressive intrahepatic cholestasis due to non-suppurative presumably autoimmune destruction of septal and the larger interlobular bile ducts. In controlled trials neither penicillamine nor azathioprine have unequivocally improved survival or slowed the progression of the disease. Because certain other autoimmune diseases appear to respond favorably to alkylating agents but not to azathioprine, a controlled trial of chlorambucil therapy for patients with PBC has been initiated. Twenty-four patients (23 women, 1 man; ages 34-63) have been admitted to this trial: 13 were randomized to chlorambucil therapy and 11 to the control group. All patients have been followed for at least two years and some for as long as five years. Two of the patients in the control group but none of the treated patients have died. Serum bilirubin levels have remained stable in treated patients, but have remained markedly elevated in most of the untreated patients. IgM levels have decreased significantly in the treated patients but have risen in the untreated patients. Liver biopsies have revealed a marked diminution in hepatic inflammation among the treated patients.

PHS 6040 (Rev. 1/83) GPO 895-100

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 53,512-05 DDB

PERIOD COVERED

October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of the Uptake of Lipoproteins by Hepatic Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

E. Anthony Jones, M.D. Chief, Liver Diseases Section A:DDB

COOPERATING UNITS *(if any)*

Molecular Diseases Branch, NHLBI

LAB/BRANCH

Digestive Diseases Branch

SECTION

Liver Diseases Section

INSTITUTE AND LOCATION

NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study is inactive.

PHS 6040 (Rev. 1/83) GPO 895-100

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AM 53,513-04 DDB

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of the Role of Chemotaxis in the Pathogenesis of Hepatic Fibrosis

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
E. Anthony Jones, M.D. Chief, Liver Diseases Section A:DDB

COOPERATING UNITS *(if any)*
Laboratory of Developmental Biology and Anomalies, NIDR

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study is inactive.

PHS 6040 (Rev. 1/83) GPO 895-100

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AM 53,514-01 DDB

PERIOD COVERED
October 1, 1983 through September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Immunological studies in chronic hepatocellular disease

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
Jay H. Hoofnagle, M.D. Senior Investigator A:DDB

COOPERATING UNITS *(if any)*
Department of Pathology, Uniformed University of Health Sciences,
Bethesda, Maryland

LAB/BRANCH
Digestive Diseases Branch

SECTION
Liver Diseases Section

INSTITUTE AND LOCATION
NIADDK, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2	1.5	.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The role of immunologic mechanisms in the perpetuation of chronic viral hepatitis is being studied and the effects of different therapies for chronic viral hepatitis on the immune system are being evaluted. The role of interferon in the etiology and treatment of chronic viral hepatitis is also being assessed. Alpha interferon production by lymphocytes of patients with untreated chronic type B hepatitis is decreased and when such patients are treated with high doses of interferon alpha inteferon production by lymphocytes is decreased further. When a subgroup of patients with chronic type B hepatitis, whose lymphocytes synthesize appreciable quantities of immunoglobulins and are particularly responsive in proliferation assays, are treated with interferon serum markers of hepatitis B viral replication disappear. Interferon therapy in responders was associated with a decrease in immunoglobulin production by lymphocytes. Receptors for inteferon on peripheral blood mononuclear cells are being characterized.

PHS 6040 (Rev. 1/83) GPO 895-10

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 54016-09 DDB

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Lipoprotein Composition in Pima Indians Compared to other Racial Groups

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

B.V. Howard, Associate Chief, PCRS, NIADDK, NIH

P.H. Bennett, Chief, EFSB, NIADDK
W.C. Knowler, Chief, SFSS, NIADDK
D.J. Pettitt, Assistant Chief, SFSS, NIADDK
M-R. Taskinen, Visiting Scientist, PCRS, NIADDK

COOPERATING UNITS *(if any)*

E. Nikkila, M. Taskinen, Univ. of Helsinki, Finland; J. Fuller and M. Mattock, Guys Hospital, London, England

LAB/BRANCH

Digestive Diseases Branch

SECTION

Phoenix Clinical Research Section

INSTITUTE AND LOCATION

NIADDK, NIH, Phoenix, Arizona

TOTAL MAN-YEARS: .9	PROFESSIONAL: .2	OTHER: .7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Both low density lipoproteins (LDL) and very low density lipoproteins (VLDL) have been implicated as positive risk factors in the genesis of atherosclerosis, whereas high density lipoprotein (HDL) concentration is an independent negative risk factor. Both diabetes and obesity are associated with changes in plasma lipoproteins and these changes may be related to the increased prevalence of cardiovascular disease. We are studying lipoprotein composition in obese and diabetic Pima Indians. Comparison of data on nondiabetic Pimas to those of Caucasians indicates Pima males and females have lower total and LDL cholesterol. Pima males and females have lower HDL cholesterol than Caucasians, and there is no sex difference in HDL in the Pimas. Measurements of total cholesterol and triglyceride and HDL cholesterol were also performed on samples from 2 South Pacific populations - the Polynesians of Rarotonga and the Melanesians of New Hebrides. In both, HDL cholesterol was low, and, as in the Pimas, there were no sex differences in HDL cholesterol. Cholesterol and triglyceride in Raratongans was higher than in the population of New Hebrides, and there appeared to be a relation between the occurrence of hyperlipemia and increased cardiovascular disease (CVD) in Raratonga. The data to date on lipoprotein composition of these various populations suggest there are racial differences in lipoprotein distributions which may be important in the determination of the prevalence of CVD. Diabetes in Pimas was associated with increases in VLDL triglyceride and decreases in HDL cholesterol in both sexes at all ages. Changes in lipoprotein distribution were greater in female diabetics, because LDL cholesterol was increased. Multiple regression analyses indicated that plasma glucose appeared to be the most important determinant of both VLDL and HDL in diabetics.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 54018-08 DDB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Metabolic and Morphologic Abnormalities in Diabetes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: C. Bogardus Acting Chief PCRS, NIADDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Digestive Diseases Branch

SECTION

Phoenix Clinical Research Section

INSTITUTE AND LOCATION

NIADDK, NIH, Phoenix, Arizona 85016

TOTAL MAN-YEARS: 0	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM54019-08 DDB

PERIOD COVERED
October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cell Metabolism on Cultures from Diabetic and Nondiabetic Subjects

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

B.V. Howard, Associate Chief, PCRS, NIADDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIADDK, Phoenix, Arizona

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Elevated insulin levels and insulin resistance in Pima Indian diabetics and nondiabetics suggest that cellular changes external to the pancreas may be important in the pathogenesis of obesity and diabetes mellitus. The aim of this project was to study several aspects of insulin function in cultured cells obtained from normal and diabetic Pima Indians, in order to determine if diabetes mellitus is expressed in genetic differences in insulin action at the cellular level. Fibroblast cultures have been established from normal and diabetic Pima Indians. Cellular metabolism and transport function were studied in the presence and absence of insulin, in order to define cell insulin action in this system. A system was also developed to measure glucose transport in endothelial cell cultures derived from umbilical veins, and insulin was observed to stimulate transport in growing cells but not in confluent monolayers. No differences were observed in any of the measurable parameters of insulin action in cells derived from diabetics, suggesting that there may not be intrinsic differences in cellular response to insulin in type 2 diabetics. Since there were no observed differences in this system relevant to the changes which occur in vivo in diabetics, this project has been terminatd.

TERMINATED

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 54021-07 DDB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Hemoglobin A1 Levels in Diabetes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J. E. Foley	Staff Fellow	PCRS, NIADDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Digestive Diseases Branch

SECTION

Phoenix Clinical Research Section

INSTITUTE AND LOCATION

NIADDK, NIH, Phoenix, AZ

TOTAL MAN-YEARS: 0	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has been terminated

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 54023-07 DDB

PERIOD COVERED
October 1, 1983 - September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Lipoprotein Metabolism in Pima Indians

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

B.V. Howard, Associate Chief, PCRS, NIADDK
M-R Taskinen, Visiting Scientist, PCRS, NIADDK
S. Lillioja, Staff Fellow, PCRS, NIADDK

COOPERATING UNITS (if any)
S.M. Grundy, and W. Beltz, Dept. of Medicine, U of TX S.W. Medical School, Dallas; G.M. Reaven, Dept. of Medicine, Stanford U., Palo Alto, CA

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIADDK, Phoenix, Arizona

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.8	.4	1.4

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Pima Indians have a high prevalence of obesity, diabetes mellitus and hyperinsulipemia, but they have low plasma cholesterol levels, reduced low density lipoprotein (LDL) synthesis, and decreased incidence of cardiovascular disease. Lipoprotein metabolism in Pima Indians is being investigated in order to further understand control of lipoprotein metabolism in this population and how lipid metabolism is influenced by obesity and diabetes.

Simultaneous studies have been performed of very low density lipoprotein (VLDL) apoB and LDL apoB metabolism using a double isotope procedure. Methods have been developed to facilitate isolation of apoB and estimation of plasma volume. In addition, a multicompartmental model has been developed for the analysis of kinetic data for the simultaneous measure of VLDL, IDL and LDL apoB metabolism. Studies in a group of male Pimas indicated that obesity results in a greatly increased flux of VLDL apoB. LDL apoB levels were not elevated in the obese subjects, because of both increased metabolism of VLDL through the alternate pathway and increased catabolism of LDL. Comparison of apoB metabolism in Pimas and weight-matched Caucasians indicated that the low LDL in Pimas is related to both higher FCR for LDL and higher proportion of VLDL metabolized without conversion to LDL. Furthermore, VLDL "shunt" and LDL FCR appeared to be correlated. The results of lipoprotein metabolic studies in Pimas to date suggest a large flux of substrates and regulators (i.e. insulin) which cause overproduction of lipoproteins, but that compensatory mechanism are operative which result in maintenance of low plasma concentrations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 54024-07 DDB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
In Vitro Insulin Resistance in Pima Indians

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J.E. Foley	Senior Staff Fellow	PCRS, NIADDK
Others:	C. Bogardus	Chief	PCRS, NIADDK
	J. Zawadzki	Clinical Fellow	PCRS, NIADDK
	S. Lillioja	Clinical Fellow	PCRS, NIADDK
	W. Abbott	Clinical Fellow	PCRS, NIADDK
	A. Young	Clinical Fellow	PCRS, NIADDK
	S. Cushman	Chief	CMOS, NIADDK

COOPERATING UNITS (if any)

Gerald Reaven, Stanford University and V.A. Hospital, Palo Alto, CA

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIADDK, NIH, Phoenix, Arizona 85016

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.8	0.6	1.2

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are currently measuring insulin binding, glucose transport, glucose metabolism, and lipolysis in adipocytes isolated from human subcutaneous abdominal tissue of Pima Indians. Subjects with normal glucose tolerance and with two nondiabetic parents or one diabetic parent have also been studied. In addition, subjects with glucose intolerance or diabetes have been studied as part of a cross sectional study in the Pima Indians. We have shown that adipocytes from diabetic subjects have altered adipocyte metabolism (i.e. decreased glucose transport and metabolism and increased basal lipolysis without any change in insulin binding). We have also shown that the capacity defects, but not the sensitivity defects, can be improved with improved glycemia via insulin therapy. In the current year we have now determined that sensitivity of glucose transport to insulin (1/ED50) in subjects with abnormal but not impaired glucose tolerance were almost half of that found in subjects with normal glucose tolerance. The subjects were matched for age, sex, obesity, fasting glucose and insulin concentrations and adipose cell size. There were no differences in capacity of transport, metabolism or lipolysis, insulin binding, as well as the sensitivity of antilipolysis to insulin. Thus, the sensitivity of glucose transport to insulin appears to be lower in subjects with abnormal glucose tolerance, due to a post binding defect in the coupling of insulin binding to glucose transport which preceeds the glycemia associated with NIDDM. This may explain why this sensitivity defect was not improved with improved glycemia while the other defective parameters of adipocytes metabolism associated with NIDDM were improved. We also are attempting to determine whether the differences in parental diabetic status influence the parameters of glucose metabolism independent of changes in glucose tolerance. We are comparing in vitro adipocyte data with the in vivo data (see project #Z01 AM 54034-02 DD) to determine whether the changes in in vitro adipocyte metabolism parallel those of in vivo glucose metabolism.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AM 54028-05 DDB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Determination of Gastric Acid Secretion in Pima Indians

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Clifton Bogardus, Acting Chief, PCRS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIADDK, NIH, Phoenix, Arizona 85016

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
0	0	0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The findings of low acid production, hypergastrinemia, and slow emptying of the stomach suggests that hypochlorhydria may be an important reason for the rarity of duodenal ulcer in Pima Indians. No changes in liquid gastric emptying rates were found in obesity. The hyperinsuinemia of obesity also did not appear to be mediated by the gastric emptying rate or by circulating plasma SLI levels. Hypergastrinemia was observed to occur in Type II diabetes, apparently as a result of autonomic dysfunction with slow gastric emptying.

This project has been terminated.

TERMINATED

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM 54029-04 DDB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Nutrition induced alterations in metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Clifton Bogardus, Acting Chief, PCRS, NIADDK

OTHERS:		
	E. Danforth	Dept. of Medicine, U of VT
	E.A.H. Sims	Dept. of Medicine, U of VT
	E. Horton	Dept. of Medicine, U of VT
	L. Lansburg	Dept. of Medicine, Harvard University
	J. Young	Dept. of Medicine, Harvard University

COOPERATING UNITS *(if any)*
Department of Medicine, University of Vermont, Burlington VT
Department of Medicine, Harvard University, Boston, MA

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIADDK, NIH, Phoenix, Arizona 85016

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0	0.1

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A "thrifty gene" has been proposed as a genetic defect that would predispose the Pima Indians to store energy very efficiently and account for their high prevalence of obesity. To determine if such genetic differences exist, the thermogenic response to three stimuli - food, norepinephrine, and exercise - were measured during a period of weight maintenance and after three weeks of overfeeding. These studies were done in lean and obese Indians. The major finding to date has been that obese Pimas, though having similar basal plasma concentrations of norepinephrine to Caucasians, achieve much higher blood concentrations of the hormone during identical norepinephrine infusions. The data suggests that obese Pima Indians have a clearance defect of norepinephrine. Turnover studies will be done to confirm these data.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 54030-04 DDB

PERIOD COVERED

October 1, 1983 - September 31, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Radioimmunoassay of polypeptide hormones

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI:	B. Vasquez	Senior Staff Fellow	PCRS, NIADDK
Others:	C. Bogardus	Chief	PCRS, NIADDK
	S. Lillioja	Clinical Fellow	PCRS, NIADDK
	W.C. Knowler	Chief	PCRS, NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH

Digestive Diseases Branch

SECTION

Phoenix Clinical Research Section

INSTITUTE AND LOCATION

NIADDK, NIH, Phoenix, Arizona 85016

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Measurement of insulin, C-peptide, glucagon, and somatostatin are central to epidemiological and metabolic studies which address the role of islet cell function and regulation and insulin resistance in the development and maintenance of obesity and diabetes in the Pima population. In vivo insulin catabolism, assessed by the hyperinsulinemic glucose clamp technique or as fractional extraction of endogenously secreted insulin, was elevated in obese diabetic patients compared to obese nondiabetic controls. The increased insulin clearance rate may contribute to the post-challenge hypoinsulinemia observed in diabetic patients. In addition, in collaboration with SFSS, NIADDK (see Z01 AM 69000-18 EFS), the cross-sectional differences and longitudinal changes in serum insulin levels in the Pima population were examined in relationship to diabetes and obesity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 54032-03 DDB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cellular Metabolism of Cultured Human Adipocyte Precursors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Vasquez	Senior Staff Fellow	PCRS, NIADDK
Others:	S. Lillioja	Visiting Associate	PCRS, NIADDK
	J. Foley	Senior Staff Fellow	PCRS, NIADDK
	B. V. Howard	Associate Chief	PCRS, NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Digestive Diseases Branch

SECTION

Phoenix Clinical Research Section

INSTITUTE AND LOCATION

NIADDK, Phoenix, Arizona

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cultured human adipocytes precursors (HAPs) are a promising culture system for investigation of inherent differences in cellular metabolism in obesity and diabetes. HAPS were established in culture from the stromal vascular fraction of collagenase digests of abdominal subcutaneous fat. In order to study an acute action of insulin on these cells, a method for measuring initial rates of glucose transport in confluent monolayers of HAPS was developed. Insulin stimulation of glucose transport was maximal in 30 min. and reversed by addition of anti-insulin antibody. Insulin stimulated glucose transport in HAPs with a mean (n=5) ED50 of 3 x 10^{-9}M insulin and the maximum insulin stimulated rated (137.5 $\pm$ 10.5 nL/sec·mg) was twice basal (63.4$\pm$9.8 nL/sec·mg). Mature adipocytes may still reflect the in vivo milieu at the time of biopsy. An acute action of insulin in HAPs has been defined that can now be compared to similar data obtained for mature adipocytes from the same biopsy. Thus, this system may provide a tool for distinguishing between environmentally and genetically determined alterations in cellular metabolism that contribute to the increased insulin resistance associated with obesity and diabetes.

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 54033-03 DDB

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathophysiology of the Adipocyte in Human Obesity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.E. Foley	Senior Staff Fellow	PCRS, NIADDK
Others:	C. Bogardus	Chief	PCRS, NIADDK
	J. Zawadski	Clinical Fellow	PCRS, NIADDK
	S. Lillioja	Clinical Fellow	PCRS, NIADDK
	B.V. Howard	Associate Chief	PCRS, NIADDK
	W. Abbott	Clinical Fellow	PCRS, NIADDK
	S.W. Cushman	Chief	CMOS, NIADDK
	L. Salans	Director	NIADDK

COOPERATING UNITS *(if any)*

Gerald Reaven, Stanford Univ. and V.A. Hospital, Palo Alto, CA

LAB/BRANCH

Digestive Diseases Branch

SECTION

Phoenix Clinical Research Section

INSTITUTE AND LOCATION

NIADDK, NIH, Phoenix, AZ 85016

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	.5	.9

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are currently characterizing the insulin resistance associated with obesity in the Pima Indians. Adipose cell size, which has long been associated with insulin resistance and obesity, is a focus of these studies. Insulin action is measured both in adipocytes in vitro and in vivo. Studies in adults indicate that cell size is distributed bimodally and that cell size is limited by some maximum size. The average cell size and obesity is related to insulin action. Larger adipocytes have smaller responses of glucose transport to insulin than smaller adipocytes, and glucose transport and antilipolysis are less sensitive to insulin and obese individuals versus lean individuals. Lipolysis is greatly increased with increasing adipose cell size. The capacity for decreased glucose transport in adipocytes is not affected by cell size or obesity. Similar findings have been found with the euglycemic clamp where the capacity (2000 μU/ml clamp) is not well correlated with obesity while the sensitivity (estimated from the 100 μU/ml clamp) is affected by obesity. Also free fatty acids levels rise in obesity. Since the in vivo glucose uptake results are reflection of insulin in muscle we have assumed that the similarities between the in vivo and in vitro studies are due similarities between muscle and fat. A major question is whether cell size changes are really important in the generation of insulin resistance associated with obesity or whether it is simply a consequence of some other factor common to fat and muscle. In an attempt to look at individuals with similar levels of obesity, but potentially different adipose cell sizes and levels of insulin resistance, we are currently studying Pima Indian children of similar obesity with family histories of NIDDM and those without. Preliminary results suggest that children of a mother diabetic during pregnancy have very large adipose cell sizes relative to children of normal caucasians of similar age and obesity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 54034-02 DDB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cross-sectional and longitudinal studies of "prediabetes" in the Pima Indians

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Principle Investigator: Clifton Bogardus, Acting Chief, PCRS, NIADDK

B.V. Howard	Associate Chief	PCRS, NIADDK
J. E. Foley,	Senior Staff Fellow	PCRS, NIADDK
D.M. Mott,	Research Chemist	PCRS, NIADDK
S. Lillioja	Visiting Associate	PCRS, NIADDK
J. Zawadzki	Medical Staff Fellow	PCRS, NIADDK
A. Young	Visiting Scientist	PCRS, NIADDK
W. Abbott	Visiting Scientist	PCRS, NIADDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Digestive Diseases Branch

SECTION

Phoenix Clinical Research Section

INSTITUTE AND LOCATION

NIADDK, Phoenix, Arizona

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
20.0	5.0	15.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Pima Indians have the highest reported prevalence and incidence of noninsulin dependent diabetes mellitus (NIDDM) of any population in the world. The diabetes occurs more frequently in offspring of a diabetic mother than in offspring of nondiabetic parents. The reasons for this are unknown. In this study we have planned to determine the metabolic abnormality that predisposes the former group of offspring to develop NIDDM. Due to the extremely high incident rate of NIDDM in this population a sufficient number of offspring of diabetic mothers will develop NIDDM within five years such that it will be possible to determine which metabolic parameter, if any, is predictive of NIDDM in this population. We will be studying many aspects of carbohydrate metabolism, both in vivo and in vitro, in an initial cross-sectional comparison and a longitudinal study. To date, approximately 200 subjects have been enrolled in the initial cross-sectional phase of this project. Approximately 60 subjects have returned for their first yearly follow-up studies. Considerable data has already been collected. Increasing obesity is only weakly associated with evidence of a "post-receptor defect" in insulin action in vivo and in vitro in isolated abdominal adipocytes. Surprisingly there are only very weak correlations between maximal insulin stimulated glucose transport in isolated adipocytes and the maximal insulin stimulated glucose disposal rate in vivo. The maximal oxygen uptake appears to account for about 25% of the observed variance of insulin action at lower high insulin concentrations. At lower insulin concentrations, obesity appeared to be more correlated with insulin action in vivo but again could only account for a very small amount of the variance observed in in vivo insulin action. Mildly glucose intolerant subjects compared to normal glucose tolerant subjects appear to have a increased ED 50 for insulin stimulated glucose transport in isolated adipocytes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 54035-02 DDB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effects of exercise with muscle glycogen depletion on in vivo insulin action

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Clifton Bogardus, Acting Chief, PCRS, NIADDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Digestive Diseases Branch

SECTION

Phoenix Clinical Research Section

INSTITUTE AND LOCATION

NIADDK, Phoenix, Arizona

TOTAL MAN-YEARS: 1.0	PROFESSIONAL: .1	OTHER: .9

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In rats, muscle glycogen depletion has been associated with increased insulin action. Whether this also occurs in man has not been reported. After four days of rest, thirteen males (E group) had a percutaneous muscle biopsy of the vastus lateralis muscle before and after a euglycemic clamp at plasma insulin concentrations of approximately 100 and 1900 μU/ml, with simultaneous indirect calorimetry. This was repeated one week later, but after glycogen depleting exercise the night before the euglycemic clamp. Seven subjects underwent the same protocol but were also refed 100 grams of carbohydrate after the excerise (EF group). In both groups the mean muscle glycogen content was approximately 40% lower after exercise compared with the muscle glycogen content measured after rest. In the E group, the mean muscle glycogen synthase activity increased three-fold after exercise, but increased only two-fold in the EF group. In both groups the basal and insulin stimulated glucose oxidation rates were lower in the post exercise condition. The insulin stimulated glucose storage rate increased significantly in the E group after exercise but not in the EF group. In the E group, total insulin mediated glucose disposal (M) was 17% and 10% higher after exercise during the low and high dose insulin infusion, respectively. No significant changes in M were observed in the EF group.

For all subjects M correlated with the carbohydrate storage rates during the low and high dose insulin infusion. In the E group the mean muscle glycogen synthase activity increased three-fold after exercise but increased only two-fold in the EF group. We concluded that muscle glycogen depletion is associated with increased in vivo insulin action in man. The increased insulin action is correlated with increased muscle glycogen synthase activity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 54036-02 DDB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Control of glucose transport in insulin sensitive tissues

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J.E. Foley	Senior Staff Fellow	PCRS, NIADDK
Others:	K. Kubo	Fogarty Fellow	PCRS, NIADDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Digestive Diseases Branch

SECTION

Phoenix Clinical Research Section

INSTITUTE AND LOCATION

NIADDK, NIH, Phoenix, AZ 85016

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
.5	.3	.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1. We have previously shown that 2-deoxyglucose transport was lower than that of 3-O-methylglucose transport and that a 10 mM glucose preincubation can accelerate the 2-deoxyglucose transport rate up to the 3-O-methylglucose transport rate. We now know that this acceleration can be maintained in the absence of glucose for at least 30 minutes after the removal of the glucose. We had previously shown that a 15-30 min incubation resulted in a maximum acceleration. We are now investigating the amount of time that glucose actually has to be present during this 30 min preincubation in order to obtain the 2-deoxyglucose acceleration. If this period is short (<5 min) we will investigate whether this effect is dependent upon protein synthesis.

2. Many investigators have proposed that glucose transport is only rate limiting step in glucose uptake into muscle under both physiological or patho-physiological conditions . This is based on the lack of any measureable intracellation glucose concentrations. However, we have previously shown that there can be "local" accumulation of glucose near the glucose transporter in adipocytes, and that the glucose clearance is decreased at the glucose concentrations where this accumulation occurs. In order to investigate the possibility that glucose uptake into muscle is also not rate limited by glucose transport, under all physiological or patho-physiological conditions, we are setting up the rat hindlimb perfussion technique and will measure glucose uptake into muscle over a range of glucose and insulin concentrations. We eventually will look at which metabolic steps beyond transport (i.e. oxidation, lactate production or glycogen synthesis), are rate limiting if we find glucose/insulin concentrations where transport is not rate limiting.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AM 54037-02 DDB

PERIOD COVERED
October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Effect of overfeeding on insulin mediated glucose disposal

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: D. Mott Research Biochemist PCRS, NIADDK
OTHERS: C. Bogardus, Acting Chief, PCRS, NIADDK
S. Lillioja, Visiting Associate, PCRS, NIADDK
J.E. Foley, Senior Staff Fellow, PCRS, NIADDK

COOPERATING UNITS *(if any)*

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIADDK, NIH, Phoenix, Arizona

TOTAL MAN-YEARS: 1.5	PROFESSIONAL: .2	OTHER: 1.3

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previous studies on the relationship between insulin resistance and obesity have demonstrated the advantages of weight loss and the disadvantages of weight gain. It is possible, however, that the overnutrition which preceeds obesity may be more important than the obese state for the development of insulin resistance We have examined the effect of overnutrition on the development of insulin resistance in 15 subjects with normal glucose tolerance. Insulin resistance in the periphery and the liver was assessed with the euglycemic clamp at 100 and 1800 µU/ml insulin using ^{3}H-glucose to measure hepatic glucose production. Changes in glucose storage and oxidation were measured in vivo using indirect calorimetry. The results of this study indicate the importance of acute overnutrition for the development of insulin resistance. In addition, it relates overnutrition induced decreases in glucose disposal rates to decreases in both in vivo non-oxidative glucose disposal rates determined by indirect calorimetry and muscle glycogen synthase activity measured in vitro. Insulin binding and glucose transport measured in adipocytes were not decreased by overnutrition. The results suggest that overnutrition, independent of increased body mass, might result in reduced insulin mediated glucose storage rates as observed in obese subjects and that a reduction in glycogen synthase activity could contribute to this mechanism.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 54038-02 DDB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Characterization of Urinary Protein Excretion in Pima Indians

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. Vasquez	Senior Staff Fellow	PCRS, NIADDK
Others:	C. Bogardus	Acting Chief	PCRS, NIADDK
	S. Lillioja	Visiting Assoicate	PCRS, NIADDK
	P. H. Bennett	Chief	PCRS, NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIADDK, NIH, Phoenix, Arizona 85016

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0	0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project as a separate study has been terminated. The analysis of urinary proteins by the immunonephelometric technique has been incorporated into the ongoing procedures of the projects "Diabetes mellitus and other chronic diseases of the Gila River Indian Community (NIADDK project #Z01 AM 69000-19 EFS)", and further work will be reported as part of that project.

TERMINATED

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 54039-01 DDB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Metabolic effects of weight loss in noninsulin dependent diabetics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Clifton Bogardus, Acting Chief, PCRS, NIADDK

Co-Principal Investigators: Joanna Zawadzki, Staff Fellow, PCRS, NIADDK
James E. Foley, Senior Staff Fellow, PCRS, NIADDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Digestive Diseases Branch

SECTION

Phoenix Clinical Research Section

INSTITUTE AND LOCATION

NIADDK, Phoenix, Arizona

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.1	0.7

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Recent work in our laboratory and others has suggested that the hyperglycemia of noninsulin dependent diabetic subjects is maintained by increased hepatic glucose production. We have hypothesized that hepatic glucose production is increased due to an appropriate response to the liver to increased fluxes of free fatty acids, and gluconeogenic substrates. The subjects are also characterized by reduced insulin action and reduced peripheral insulin concentrations. In vitro evidence collected in isolated adipocytes has also suggested that there is reduced maximal insulin stimulated glucose transport rates in these individuals, along with increased basal lipolytic rates. In order to define which of these abnormalities might be most responsible for the hyperglycemia of the diabetics we plan a weight loss study to correlate changes in fasting glycemia with changes in these other aspects of carbohydrate metabolism in vivo and in vitro. Noninsulin dependent diabetic subjects with fasting glycemias greater than 200 mg/dl and BMIs greater than 30 will be admitted to the clinical research ward. After 10 days of weight maintainence and adaptation to the research ward diet, studies of body composition, insulin secretion, insulin action, hepatic glucose production and glucose recycling through the Cori cycle, and measurements of abdominal adipocyte metabolism will be made. Subjects will then eat a weight losing diet, calculated as 30% of their weight maintenance requirements, for up to 5 1/2 weeks. This will be followed by another weight maintainence diet of approximately 10 days when the above studies will be repeated. Our hypothesis is that reduction in glycemia associated with reduction in body weight will be best correlated with decreases in hepatic glucose production and glucose recycling through the Cori cycle.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 54040-01 DDB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Relationships between insulin secretion, action, and glucose concentrations

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Clifton Bogardus, Acting Chief, PCRS, NIADDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

Digestive Diseases Branch

SECTION

Phoenix Clinical Research Section

INSTITUTE AND LOCATION

NIADDK, Phoenix, Arizona

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.3	.2	1.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Uncertainty remains regarding the relative contribution of either abnormal insulin secretion or insulin action to the pathogenesis of noninsulin dependent diabetes mellitus. In this study we have examined the relationships between insulin secretion, insulin action and fasting glycemia in nondiabetic and noninsulin dependent diabetic southwest American Indians. We have quantified the insulin secretion by measuring the insulin and C-peptide responses after oral glucose and after a 24 hour standard diet of mixed composition. In vivo insulin action was estimated using the euglycemic clamp technique, with simultaneous indirect calorimetry, at both submaximal and maximal insulin stimulating concentrations.

The results suggest that moderately increased fasting glycemia in nondiabetics, and in mildly hyperglycemic noninsulin dependent diabetics was primarily a result of a decrease in insulin action. Marked hyperglycemia in the diabetics occurred as a result of both decreased insulin action and decreased plasma insulin concentrations. Decreased plasma insulin concentrations appeared to be a result both of insufficient insulin secretion and increased insulin disappearance rates. We have hypothesized that the major results of decreased insulin action and decreased plasma insulin concentrations in the diabetics subjects were increased free fatty acid concentrations, increased fat oxidation rates, and increased flux of gluconeogenic substrates to liver, leading to increased glucose production and progressive hyperglycemia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AM 54041-01 DDB

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Sulfonylurea therapy and lipid metabolism in Type II diabetes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Barbara V. Howard, Associate Chief, PCRS, NIADDK

Others: Clifton Bogardus, Acting Chief, PCRS, NIADDK
Stephen Lillioja, Visiting Scientist, PCRS, NIADDK
William Abbott, Visiting Scientist, PCRS, NIADDK

COOPERATING UNITS *(if any)*
3rd Dept. of Medicine, University of Helsinki School of Medicine, Helsinki
Department of Medical Education, Good Samaritan Medical Center, Phoenix, AZ

LAB/BRANCH
Digestive Diseases Branch

SECTION
Phoenix Clinical Research Section

INSTITUTE AND LOCATION
NIADDK, Phoenix, Arizona

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
2.0	.5	1.5

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The hypertriglyceridemia commonly associated with type II diabetes is of concern because of its possible role in the etiology of the greatly increased cardiovascular disease in this disorder. Moreover it has not been established how therapy of diabetes, especially using oral hypoglycemics, influences lipoprotein metabolism and lipase activities. This study compares VLDL metabolism, FFA metabolism in lipoprotein lipase activities in type II diabetics before and after therapy with the oral hypoglycemic agent tolinase. Results show that elevation in plasma free fatty acids in type II diabetics is caused by both increased production and impaired removal of free fatty acids. The latter was related closely to glycemic control suggesting there may be impaired reesterification of free fatty acids in uncontrolled diabetes. The results also showed that improvement of glycemic control was followed by significant falls in VLDL-TG and LDL cholesterol but no changes in HDL and HDL subfractions. The treatment of glycemia appeared to have independent effects on the metabolism of VLDL triglyceride and VLDL apoB, since in the former the transport was decreased after treatment whereas for the latter transport was unchanged whereas fractional catabolic rate increased.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 54042-01 DDB

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Relationship between insulin-mediated glucose disposal and lipid metabolism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Co-Principal Investigators:
Stephen Lillioja, Visiting Associate, PCRS, NIADDK
Barbara V. Howard, Associate Chief, PCRS, NIADDK
OTHERS: Clifton Bogardus, Acting Chief, PCRS, NIADDK
James E. Foley, Senior Staff Fellow, PCRS, NIADDK

COOPERATING UNITS (if any)

LAB/BRANCH

Digestive Diseases Branch

SECTION

Phoenix Clinical Research Section

INSTITUTE AND LOCATION

NIADDK, Phoenix, Arizona

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
1.0	.2	.8

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

It is well known that the availability of fatty acids for oxidation at the cellular level might be responsible for alterations in carbohydrate metabolism. While some in vivo studies have demonstrated that changes in plasma free fatty acids are accompanied by reciprocal changes in whole body glucose oxidation it has not been fully established how insulin mediated glucose disposal may be related to changes in endogenous lipid metabolism in man. This study combines the techniques of the euglycemic clamp, indirect calorimetry, and labelled free fatty acid infusion in order to examine the relationship between insulin mediated glucose disposal, carbohydrate oxidation and storage and measures of lipid metabolism including free fatty acid turnover and lipid oxidation. In vitro adipocyte lipolysis was also assessed from adipocytes isolated from an open fat biopsy from the same subjects. Subjects were studied over a wide range of obesity in order to provide a range of lipid metabolic profiles and insulin resistance. The data suggested direct interaction between lipid oxidation and glucose oxidation in vivo. However this interrelationship can only partially explain impaired in vivo insulin mediated glucose disposal, since the disposal of glucose involves a storage component which is of at least equal importance to oxidation and is not directly related to fatty acid metabolism.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AM 54043-01 DDB

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Muscle glycogen synthase activity and insulin mediated glucose disposal

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. Bogardus	Chief	PCRS, NIADDK
Others:	D. Mott	Research Biochemist	PCRS, NIADDK
	A. Young	Clinical Fellow	PCRS, NIADDK
	S. Lillioja	Clinical Fellow	PCRS, NIADDK

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Digestive Diseases Branch

SECTION

Phoenix Clinical Research Section

INSTITUTE AND LOCATION

NIADDK, NIH, Phoenix, Arizona

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	.2	0.7

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Most of an oral glucose load is taken up by muscle in man. Animal models for insulin resistance have reduced muscle glycogen synthase activity. In an effort to determine the importance of this enzyme to insulin mediated glucose disposal in man, we have measured the changes in muscle glycogen synthase activity with acute and chronic changes in insulin mediated glucose disposal. Following glycogen depletion both glycogen synthase activity and glucose disposal rates increased. Following overnutrition both glycogen synthase activity and glucose disposal rates decreased. In a study of subjects which were glucose tolerant or had varying degrees of glucose intolerance, insulin mediated glucose disposal rates correlated with the glycogen synthase activity measured after insulin infusion. These data indicate a possible importance of regulation of glycogen synthase activity to insulin mediated glucose disposal rates.

ANNUAL REPORT OF EPIDEMIOLOGY AND FIELD STUDIES BRANCH

NATIONAL INSTITUTE OF ARTHRITIS, DIABETES, AND DIGESTIVE AND KIDNEY DISEASES

Overview

The main goals of the Epidemiology and Field Studies Branch and its component sections concern the identification and understanding of the determinants of non-insulin-dependent diabetes mellitus (NIDDM) and its complications. Most of the studies are conducted among the Pima Indian population of Arizona. The Branch works in close collaboration with the Phoenix Clinical Research Section whose activities center around subsets of patients identified as a result of the epidemiological studies conducted by the Southwestern Field Studies Section. The Biometry Section supports the data processing and statistical analysis functions of the Phoenix Clinical Research Section and the Southwestern Field Studies Section.

Genetic Determinants of Diabetes

The genetics of NIDDM are still not known. Recently interest has centered around the presence of restriction fragment length polymorphisms in the region of the insulin gene, but extended studies have failed to reveal an association between the class 3 insertions and NIDDM in the Pima Indians. Examination of the clinical correlates of the class 3 insertions, however, has suggested that there may be an effect on the severity of the disease, as reflected by the need for insulin and oral hypoglycemic agents, but it is now clear that this particular insertion is not associated with the major genetic determinant of NIDDM in the Pima Indians.

Segregation analysis of large Pima Indian families indicates that the inheritance of diabetes in the Pima is best explained by the presence of a single major dominant gene with variable penetrance. Evidence of a single gene effect emerged initially from the presence of bimodality in glucose frequency distributions in the population. Now the presence of three and four generation families has enabled the performance of segregation analysis which appears to exclude the possibility that the disease is the result of recessive genes. A single major gene which exhibits dominance and which appears to be associated with a dosage effect, but whose penetrance varies appreciably with age, appears to be the critical determinant.

As a result of this finding efforts have been initiated to utilize existing genetic markers to perform linkage analyses to identify the chromosome which carries this genetic determinant. In addition to red cell antigens and serum protein markers, serum and red cell hemolysates have been collected to extend the bank of available markers with known chromosome locations. These together with data on the restriction fragment length polymorphism on chromosome 11 and HLA antigens (chromosome 6) will be used to conduct extensive linkage analysis. Chromosomes which are not represented with sufficient polymorphic markers will subsequently be examined using DNA probes for other restriction fragment length polymorphisms until the chromosome containing the diabetes gene is identified. Subsequently, we hope to examine other markers on this chromosome, and by means of recombination frequencies, identify the approximate location of the gene.

With the currently available technology and the use of linkage analysis in the extended Pima families, it should be possible to identify the gene location, and perhaps in the future, the structure of the diabetes gene in the Pima Indian population.

As a byproduct of the measurements of genetic markers we have investigated the possible origin of the Pima Indian group in two ways. Gm polymorphisms in the Pima have been compared with those of the number of other American Indian tribes examined in our earlier studies. These polymorphisms are consistent with the three migration hypothesis for the origin of American Indians and indicate that the Pima along with the Papago, the Puebloan, and Pai Indians are descendents of the Paleo-Indians, whose descendents now are mainly found in South America, and suggest that the most likely homeland for all three ancestral Indian populations is to be found in northeastern Asia. In addition, mitochondrial DNAs (mtDNAs) of the Pima have been examined for restriction site variants. Present in high frequency (more than 30%) in the Pima, are mtDNA variants which have been found previously only in Asians, but in much lower frequencies. These findings again point to an Asian origin of the Pima. The extent of variation in mtDNAs and the fact that these are inherited only through female lines suggest that founder effects occurred in the formation of American Indian tribes, and demonstrate that this technology will likely prove to be powerful in pinpointing the origins and interrelationships between the tribes.

Characteristics Preceding the Development of Diabetes

Another substantial area of investigation relates to abnormalities which precede the appearance of diabetes. During the past year we have focused on the importance of the intrauterine environment on the development of diabetes in the Pima since it was demonstrated that the offspring of women diabetic at the time of pregnancy had much higher rates of diabetes than those women who subsequently developed diabetes or of women who have not yet developed diabetes. The paternal (genetic) contribution has been examined and has been shown to be independent, but interactive with the diabetes status of the mother. Nevertheless, the intrauterine environment appears to have an effect which leads to the early appearance of noninsulin-dependent diabetes in the offspring of the diabetic pregnancy. This finding contributes to the higher frequency of diabetes in the offspring of diabetic mothers, which we have reported previously, and suggests that the infant may be conditioned to develop diabetes at the time of birth. Possible mechanisms for this conditioning have been suggested and will be explored in the future.

The events which precede the development of diabetes include thickening of the muscle capillary basement membrane. A followup study of genetic "prediabetic" Pima Indians and controls is being conducted to determine at what stage in the development of diabetes such thickening occurs and what are its possible determinants.

Complications of Diabetes

The Branch has continued to study several aspects of the complications of diabetes. In particular, in collaboration with the State University of New York at Buffalo, an extensive study of dental and periodontal disease has been performed. The data obtained to date indicate that diabetics are edentulous six times as frequently as the nondiabetics and that periodontal disease is much more severe among the diabetic and those with impaired glucose tolerance than among those with normal glucose tolerance. Thus, this previously little studied aspect of diabetes appears to give rise to appreciable morbidity.

Additional methods have been developed which we believe will contribute to our understanding of the natural history and development of the vascular complications of diabetes. Simplified methods for obtaining photographic assessments of retinopathy have been instituted, along with quantitative methods to measure the presence of subclinical proteinuria. In addition, methods to measure glycosylated hemoglobin and whole blood proteins have been investigated in collaboration with the University of Missouri, and it has been shown that blood collected on filter paper can be effectively used to estimate glycemic control. These methods will provide important tools for the ongoing investigations of the determinants of the vascular complications of diabetes, as well as being important instruments for clinical trials relating to the prevention of diabetes complications and the prevention of decompensation in subjects at high risk of development of diabetes.

Other Activities

The professional staff of the Branch continue to serve important roles in national and international activities. These include serving on the editorial boards of several scientific journals and serving as advisors to other Institutes, the National Center for Health Statistics, and other organizations such as the World Health Organization.

SOUTHWESTERN FIELD STUDIES SECTION

The Southwestern Field Studies Section has continued to investigate the determinants of diabetes and its complications during a longitudinal study of the natural history of diabetes in the Pima Indian population.

Further studies have been made in the causes of diabetes. While pedigree analyses suggest the existence of a major diabetes-susceptibility gene, the locus of such a gene has not yet been identified. Evidence for a genetic contribution to diabetes, evidence that hyperinsulinemia (relative to glycemia and obesity) predicts worsening glucose tolerance and that the intrauterine environment plays a role in the subsequent development of obesity and diabetes in the offspring, and our previous findings of a strong relationship of obesity and diabetes development emphasize the complex set of factors which predispose to diabetes mellitus.

Complications of diabetes remain important areas of study. Improved methods of measuring proteinuria and of assessing long-term glycemic control have been implemented, and a new retinal photographic method will be used in further studies of the development of microvascular disease in diabetics. The collaborative study on periodontal disease has progressed well. Periodontal disease appears to be strongly influenced by diabetes and impaired glucose tolerance. The finding that lipoprotein concentrations are different in diabetic and nondiabetic Pimas may help in understanding the increased incidence of macrovascular disease in diabetic Pimas. In the future, we will do further studies of risk factors for macrovascular disease in diabetics, a major cause of morbidity and mortality among diabetics in the general U.S. population.

Studies continue on the natural history of rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis. Biennial examinations include physical examinations of the joints, radiography, serology, and collection of other clinical data.

Collection and analysis of tissues from autopsied subjects and of information on cause of death on all deceased subjects continues. Age-specific death rates in Pima Indians are about twice those in the State of Arizona. Many of the deaths are due to diabetes and hepatobiliary diseases, areas of direct concern to the Institute. The 66% increase in 15-year mortality among subjects with gallstones discovered by an x-ray survey suggest that, at least in this population, silent gallstones are not a benign condition.

In view of the high prevalence of diabetes, diabetes complications, cholelithiasis, eye diseases, and periodontal disease, and high mortality, the Pima Indians continue to suffer from a heavy burden of ill health and remain a valuable study population for continued medical research.

The Section staff continue to play roles in medical research and education beyond the projects described here. Staff contribute to education through lectures and consultation with the Indian Health Service and medical schools. Epidemiologic experience gained in the major activities of the Section is applied through staff participation in research projects conducted by the Phoenix Clinical Research Section of NIADDK, the National Center for Health Statistics and the National Institute of Child Health and Human Development.

BIOMETRY SECTION

The Biometry Section serves as a resource to the Epidemiology and Field Studies Branch, the Southwestern Field Studies Section (SFSS), and the Phoenix Clinical Research Section (PCRS). The Section provides computer services and statistical consulting to these components. The scientific accomplishments are reported in the corresponding Section reports.

The most significant undertaking this year has been the enlistment of help of the Data Management Branch (DMB), DCRT, to construct a complete new data base management system for the entire Phoenix operation data base, including all data collected by SFSS and by PCRS. The system will be patterned after the DMB-supported system used in the Clinical Center. Development is well underway, with completion anticipated in mid- or late-1985.

A innovative WYLBUR Command Procedure System has been written for the automatic scheduling of insulin radioimmunoassays. Responding to specifications for the run desired (e.g., all existing samples for a subject; all samples for a given test; etc.), the systems identifies the existence and physical (freezer) location of candidate samples, avoids conflicts with other assays being run the same day, optimizes the sample selection by minimizing the risk of inter-assay variation on the same subject, and specifies the exact sample tubes for one complete pass on the gamma counter.

A data base system for studying the effects of diabetes and obesity on offspring has been completely rewritten in structured SAS (Statistical Analysis System), with provision for the incorporation of two major data files not previously utilized. The data base approaches 100,000 records.

Statistical consulting needs are now being adequately addressed with the addition of contracted services of experts in the Phoenix area.

A pilot effort is underway for contracting out a major applications programming project. Further contracts for programming may be used in an effort to reduce the backlog of unmet programming requirements. In addition the applications of personal computers to increase efficiency in the Phoenix activities have been explored.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 69000-19 EFS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Diabetes Mellitus and Other Chronic Diseases in the Gila River Indian Community

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W.C. Knowler	Chief	SFSS, NIADDK
Others:	P.H. Bennett	Chief	EFSB, NIADDK
	D.J. Pettitt	Assistant Chief	SFSS, NIADDK
	M.J. Carraher	Staff Physician	SFSS, NIADDK
	J. Everhart	Staff Fellow	SFSS, NIADDK
	R.R. Little	Assistant Professor	U. of Missouri, Columbia
	M. Shlossman	Assistant Professor	State U. of N.Y. at Buffalo
	R.C. Williams	Associate Professor	Arizona State University

COOPERATING UNITS *(if any)*

IHS Hospitals, Sacaton & Phoenix, AZ: Ariz. State University; West Virginia University; Washington University; State University of New York at Buffalo: Biometry Section, EFSB, NIADDK: Phoenix Clinical Research Section, DDB, NIADDK

LAB/BRANCH

Epidemiology and Field Studies Branch

SECTION

Southwestern Field Studies Section

INSTITUTE AND LOCATION

NIADDK, NIH, Phoenix, Arizona 85014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.2	2.5	1.7

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Evidence for a strong familial component to diabetes in the Pima Indians continues to accrue. Some of the familial influence appears to be transmitted not genetically, but via the intrauterine environment of the pregnant diabetic woman. Despite evidence suggesting the existence of at least one locus for a major diabetes susceptibility gene, evidence for association or linkage with a specific locus remains weak, with the HLA-A locus remaining the best candidate. Although genetic variation at a locus near the insulin gene had previously been shown to have a modest association with non-insulin-dependent diabetes in three racial groups, further studies of this locus suggest there is no role for this gene in diabetes in the Pimas, with the possible exception of homozygosity for one class of alleles. The distributions of other genetic markers have been described and compared with other populations and will be investigated for possible diabetes associations.

Improved methods for assessing average glycemia by glycosylated hemoglobin and blood proteins and for assessing diabetic nephropathy by a sensitive immunonephelometric measure of proteinuria have been implemented. These will be used to assess the role of hyperglycemia and other factors in the development of microvascular complications of diabetes.

Preliminary analyses suggest that periodontal disease may be much more common and severe in diabetic subjects. Clinically diagnosed gallbladder disease is more common in those with impaired glucose tolerance or diabetes than in those with normal tolerance.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 69001-15 EFS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Complications and Outcome of Diabetic and Prediabetic Pregnancies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	D.J. Pettitt	Assistant Chief	SFSS, NIADDK
Other:	P.H. Bennett	Chief	EFSB, NIADDK
	H.R. Baird	Acting Chief	BS, NIADDK
	W.C. Knowler	Chief	SFSS, NIADDK
	M.J. Carraher	Staff Physician	SFSS, NIADDK
	K.A. Aleck	Assistant Professor	Dept. of Pediatrics, U. of Illinois

COOPERATING UNITS *(if any)*

Indian Health Service; Department of Pediatrics, University of New Mexico; Biometry Section, EFSB

LAB/BRANCH

Epidemiology and Field Studies Branch

SECTION

Southwestern Field Studies Section

INSTITUTE AND LOCATION

NIADDK, NIH, Phoenix, Arizona 85014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.9	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Diabetes mellitus is a chronic disease which has deleterious effects on the progress and outcome of pregnancy. Mothers with diabetes during pregnancy are more likely to have toxemia, cesarean sections, and also have higher mortality and offspring are more likely to be congenitally malformed, macrosomic, premature, or stillborn. The effects on the offspring of women who have diabetes during pregnancy are long lasting, possibly for life. In addition, diabetes can first occur during pregnancy or occur only during pregnancy (gestational diabetes).

The purpose of the project is to determine the effects of diabetes mellitus and prediabetes on the complications and outcome of pregnancy in women in the Gila River Indian Community and on the subsequent growth and development of their children to gain a better basis for understanding mechanisms by which the diabetic pregnancy affects the offspring.

The diabetes status of every woman is determined at two-yearly intervals and during the third trimester of each pregnancy. The characteristics of women who have diabetes during the pregnancy (diabetics) are compared to those of women who are normal during the pregnancy and subsequently develop diabetes (prediabetics) or remain normal (nondiabetics). The outcome of these pregnancies is assessed by review of both mother's and infant's hospital records. The offspring of these pregnancies are followed at two-yearly intervals after the age of 5 years with examinations which include measurement of numerous parameters including glucose tolerance,. height, and weight. The diabetes status of the fathers of these offspring is also determined so that familial and genetic factors can be evaluated as well as the effects of the intrauterine environment.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 69003-11 EFS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Muscle Capillary Basement Membrane Thickness Prior to Onset of Diabetes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	P.H. Bennett	Chief	EFSB, NIADDK
Others:	N.B. Rushforth	Chairman	Dept. of Biology Case Western Reserve U.
	M.D. Siperstein	Vice Chairman	Dept. of Medicine, UCSF
	O. Famuyiwa	Fogarty Fellow	EFSB, NIADDK
	C. Bogardus	Acting Chief	PCRS, DDB, NIADDK
	W.C. Knowler	Chief	SFSS, NIADDK

COOPERATING UNITS *(if any)*

Department of Biology, Case Western Reserve University, Cleveland, Ohio; Department of Medicine, University of California, San Francisco, California; PCRS, DDB, NIADDK

LAB/BRANCH

Epidemiology and Field Studies Branch

SECTION

Southwestern Field Studies Section

INSTITUTE AND LOCATION

NIADDK, NIH, Phoenix, Arizona

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.1	1.3	0.8

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Several metabolic and morphologic changes have been claimed to precede the onset of diabetes, including changes in the pattern and quantity of insulin secretion and alteration in the thickness of capillary basement membranes. Some 10 years ago, Pima Indians with two diabetic parents, and with neither parent diabetic received oral and intravenous glucose tolerance tests, and a biopsy of the quadriceps muscle from which quantitative determinations of the thickness of the capillary basement membrane were made. The same subjects are now being reexamined to determine if their insulin secretion or the thickness of their capillary basement membrane predicted the subsequent development of diabetes, and to determine if their was differential thickening of the muscle capillary basement membrane with increasing age in those with diabetic parents compared to those without. The results will help to determine if vascular lesions at the level of the capillary are present before hyperglycemia develops.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 69006-14 EFS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Gila River Indian Community Autopsy Study

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

PI:	P.H. Bennett	Chief	EFSB, NIADDK
Other:	W.C. Knowler	Chief	SFSS, NIADDK
	M.J. Carraher	Staff Physician	SFSS, NIADDK
	D.J. Pettitt	Assistant Chief	SFSS, NIADDK
	D. Althaus	Pathologist	Phoenix Indian Medical Center
	L. Orci	Chairman	Dept. of Morphology, University of Geneva

COOPERATING UNITS *(if any)*

Pathology Department, Phoenix Indian Medical Center, Phoenix, Arizona
Dept. of Morphology, University of Geneva, Geneva, Switzerland

LAB/BRANCH

Epidemiology and Field Studies Branch

SECTION

Southwestern Field Studies Section

INSTITUTE AND LOCATION

NIADDK, NIH, Phoenix, Arizona 85014

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The postmortem characteristics of Pima Indians of the Gila River Indian Community are investigated so that findings in subjects with and without diabetes mellitus can be correlated with studies in living subjects. In particular, the histology of the pancreatic islets of Langerhans is being studied in detail to describe the changes that take place in diabetes.

A major analyses of causes of death, based on death certificates, of all Gila River Indian Community residents dying since 1970 has been undertaken. The age-adjusted mortality rates due to all causes were approximately twice the rates for the State of Arizona. Particularly high were rates of death due to trauma, infections, diabetes, respiratory diseases, alcoholism and cirrhosis. In women, the rate of death due to biliary carcinoma was over 13 times the state rate.

During a random survey for the prevalence of gallstones in 1968, 307 Pima Indians without prior evidence of gallbladder disease had oral cholecystograms (OCG). Medical records of 294 of the subjects were reviewed in 1983. Three cases of biliary carcinoma were found in 107 people with abnormal OCGs, and none were found in 160 with normal nor in 27 with uncertain OCG results. These findings are consistent with the hypothesis that gallstones are a risk factor for biliary carcinoma.

The 15-year cumulative mortality rates were examined in relation to the OCG result. Age-sex-adjusted mortality from all causes was 66% higher in those with abnormal than in those with normal cholecystograms (p less than 0.01). There were no deaths attributed to cholecystectomies. These findings suggest that silent gallstones are not a benign condition in Pima Indians, and may predispose subjects, especially Indian women, to biliary carcinoma.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 69009-19 EFS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Natural History of Arthritis and Rheumatism in the Gila River Indian Community

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.H. Bennett	Chief	EFSB, NIADDK
Other:	D.J. Pettitt	Assistant Chief	SFSS, NIADDK
	W.C. Knowler	Chief	SFSS, NIADDK
	M.J. Carraher	Staff Physician	SFSS, NIADDK
	J. Henri	Visiting Associate	EFSB, NIADDK
	H.R. Baird	Acting Chief	BS, NIADDK

COOPERATING UNITS *(if any)*

Biometry Section, EFSB

LAB/BRANCH

Epidemiology and Field Studies Branch

SECTION

Southwestern Field Studies Section

INSTITUTE AND LOCATION

NIADDK, NIH, Phoenix, Arizona

TOTAL MAN-YEARS: 1.9	PROFESSIONAL: 0.7	OTHER: 1.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The development and progression of osteoarthrosis, rheumatoid arthritis and ankylosing spondylitis are being determined by means of clinical, radiographic and serological examinations carried out prospectively at two-yearly intervals among adults of the Gila River Indian Community (Pima Indians) in Arizona, in conjunction with epidemiological studies of diabetes in the same community. The purpose of this investigation is to ascertain the determinants of these diseases in the population, and to identify factors which alter the natural history or progression of the diseases. The relationship of host factors, including various gene markers, such as HLA and Gm type, and age, sex, together with various potential environmental determinants, such as obesity, and evidence of exposure to infectious agents, to the development of these diseases will be determined prospectively.

Longitudinal data have now been collected over a 20 year period in the population, and represent a unique data-set for epidemiological studies of arthritis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM 69013-03 EFS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Diabetes, Arthritis and Other Metabolic Diseases in the Pacific Region

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.H. Bennett	Chief	EFSB, NIADDK
Others:	P. Zimmet	Director	WHO Collaborating Centre for the Epidemiology of Diabetes Mellitus, Melbourne, Australia
	H. King	Associate Director	WHO Collaborating Centre for the Epidemiology of Diabetes Mellitus, Melbourne, Australia

COOPERATING UNITS *(if any)*

WHO Collaborating Centre for the Epidemiology of Diabetes Mellitus, South Pacific Commission; PCRS, DDB, NIADDK

LAB/BRANCH

Epidemiology and Field Studies Branch

SECTION

Southwestern Field Studies Section

INSTITUTE AND LOCATION

NIADDK, NIH, Phoenix, Arizona

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The prevalence of diabetes and its associated vascular complications have been assessed in several Pacific Island populations, including Polynesians and Melanesians, living in traditional ways as well as in urbanized communities.

In general, much higher prevalences of diabetes and the associated complications were found in the urbanized populations, and attempts to determine the reasons for these differences are being pursued. Increased obesity, reduced physical activity, changes in dietary composition and intake appear to contribute to these differences in frequency, but genetic factors also are likely important in determining the frequency of the diabetes, itself, and possibly the type and frequency of associated complications. Identification of the relative importance of environmental determinants of diabetes is a prerequisite to formulating preventive measures for this disease in developing countries.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM70500-06 CMOS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Insulin's Regulation of Glucose Transport and Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. W. Cushman	Chief	CMOS, NIADDK
Others:	I. A. Simpson	Visiting Scientist	CMOS, NIADDK
	B. B. Kahn	Medical Staff Fellow	CMOS, NIADDK
	K. C. Appell	Staff Fellow	CMOS, NIADDK
	D. L. Baly	Staff Fellow	CMOS, NIADDK
	M. Kuroda	Guest Worker	CMOS, NIADDK
	M. J. Zarnowski	Biologist	CMOS, NIADDK
	D. Yver	Chemist	CMOS, NIADDK

COOPERATING UNITS *(if any)* Department of Medicine, University of California at San Diego, La Jolla, CA (R. Horuk); Department of Medicine, University of Lund at Malmo, Malmo, Sweden (U. SMith); Office of the Dean, Mount Sinai Medical School, New York, NY (L. Salans)

LAB/BRANCH

SECTION

Cellular Metabolism and Obesity

INSTITUTE AND LOCATION

NIADDK, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8.5	8.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Internalization of insulin and its receptor have been examined in rat adipose cells at 37°C. Insulin and its receptor are rapidly translocated into the cell's high- and low-density microsomes; the receptor's vectorial disposition in the plasma membrane is inverted during internalization. Acute phosphorylation of the insulin receptor is not essential for internalization or the response to insulin. Insulin stimulates glucose transport in rat adipose cells through the translocation of glucose transporters from an intracellular pool to the plasma membrane. The intracellular pool of glucose transporters comprises a specialized membrane species and intracellular glucose transporters are processed during their cycling. Insulin appears to to stimulate glucose transport specifically at a step in which glucose transporters in the plasma membrane become functional. A photoaffinity crosslinking agent has now been used to attach [^{3}H]cytochalasin B to the glucose transporter. Chronic hyperinsulinemia increases insulin binding and the capacity of rat adipose cells to transport and metabolize glucose; hyperinsulinemia enhances insulin's stimulatory action on glucose transport by increasing the intracellular pool of glucose transporters. Insulin resistant glucose transport induced by fasting is due to a depletion of intracellular glucose transporters; however, the hyperresponsive insulin-stimulated glucose transport activity associated with refeeding is not totally accounted for by a change in the number of glucose transporters. Catecholamines counterregulate insulin-stimulated glucose transport, but only in part by modulating the translocation of glucose transporters; this counterregulation appears to comprise both cAMP-independent and -dependent mechanisms. Insulin also induces an increase in IGF-II binding in isolated rat adipocytes by causing a steady state redistribution of receptors from a large intracellular pool to the plasma membrane.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM70501-06 CMOS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Insulin-Cell Interaction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	I. A. Simpson	Visiting Scientist	CMOS, NIADDK
Others:	J. A. Hedo	Visiting Associate	DB, NIADDK
	S. W. Cushman	Chief	CMOS, NIADDK
	D. R. Yver	Chemist	CMOS, NIADDK

COOPERATING UNITS *(if any)*

Institute of Physiology, University of Aarhus, Aarhus, Denmark (O. Sonne).

LAB/BRANCH

SECTION

Cellular Metabolism and Obesity

INSTITUTE AND LOCATION

NIADDK, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.9	0.9	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The time-course and insulin concentration dependency of internalization of insulin and its receptor have been examined in isolated rat adipose cells at 37°C. The results demonstrate that insulin is rapidly translocated into the cell's high- and low-density microsomes. Insulin simultaneously induces the translocation of its own receptor into the latter compartment. A protocol has been developed for maintaining isolated rat adipose cells in primary tissue culture. The acute insulin-induced internalization of its own receptor was then confirmed by biosynthetically labeling cells in culture with either [^{35}S]methionine or [^{3}H]glucosamine. The spatial orientation of the α and β subunits of the insulin receptor in the rat adipose cell plasma membrane has been examined using lactoperoxidase/Na^{125}I iodination. The results suggest that the α receptor subunit is primarily exposed on the extracellular surface of the plasma membrane while the β receptor subunit traverses the membrane, and this vectorial disposition is inverted during internalization. In a preliminary study, anti-insulin receptor antiserum B-10 induces internalization of the insulin receptor and stimulates glucose transport in the rat adipose cell, but does not appear to affect the phosphorylation state of the β receptor subunit. Thus, the acute phosphorylation of the insulin receptor induced by insulin is not essential per se for internalization of the receptor or the response to insulin. The biosynthesis of the insulin receptor has been studied in primary cultures of isolated rat adipose cells. The results suggest that insulin receptors are synthesized through the formation and processing of a 190K precursor in the endoplasmic reticulum and Golgi followed by insertion of the mature 135K and 95K subunits into the plasma membrane.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM70502-06 CMOS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Insulin's Regulation of Glucose Transport

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. W. Cushman	Chief	CMOS, NIADDK
Others:	I. A. Simpson	Visiting Scientist	CMOS, NIADDK
	B. B. Kahn	Medical Staff Fellow	CMOS, NIADDK
	K. C. Appell	Staff Fellow	CMOS, NIADDK
	D. L. Baly	Staff Fellow	CMOS, NIADDK
	M. J. Zarnowski	Biologist	CMOS, NIADDK
	D. R. Yver	Chemist	CMOS, NIADDK

COOPERATING UNITS *(if any)*

Department of Medicine, University of California at San Diego Medical School, La Jolla, CA (R. Horuk); Office of the Dean, Mount Sinai Medical School, New York, NY (L. B. Salans).

LAB/BRANCH

SECTION

Cellular Metabolism and Obesity

INSTITUTE AND LOCATION

NIADDK, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.2	3.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Insulin stimulates glucose transport in rat adipose cells through the translocation of glucose transporters from an intracellular pool to the plasma membrane. A detailed characterization of the subcellular fractions of these cells, and of the distribution of glucose transporters among these fractions is now described. The results suggest that the intracellular pool of glucose transporters may comprise a specialized membrane species and that intracellular glucose transporters may be processed during their cycling to the plasma membrane in response to insulin. The effects of TRIS on insulin's stimulation of glucose transport have been investigated. The results suggest that insulin's action on the translocation of glucose transporters may occur at a step whereby glucose transporters associated with the plasma membrane become functional. The effects of low temperature on the stimulation of glucose transport by insulin have been examined. The results confirm that insulin appears to stimulate glucose transport through a step in which glucose transporters in the plasma membrane become functional. The mechanism of insulin resistant glucose transport in isolated guinea pig adipocytes has been examined. The results suggest that this resistance is the consequence of a relative depletion in the number of intracellular glucose transporters. In a preliminary series of experiments, insulin appears to stimulate glucose transport in isolated human adipose cells by a translocation mechanism similar to that observed in rat adipose cells and diaphragm. The photoaffinity crosslinking agent hydroxysuccinimidyl-4-azidobenzoate has been used to attach [^{3}H]cytochalasin B to a rat adipocyte low-density microsomal membrane protein of 45-50 kDa. The characteristics of this protein are consistent with those of the glucose transporter. Thus, the identification, isolation, and purification of the adipocyte glucose transporter should now be possible.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 AM70503-06 CMOS

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Alterations in Insulin's Action in Obesity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. W. Cushman	Chief	CMOS, NIADDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

SECTION
Cellular Metabolism and Obesity

INSTITUTE AND LOCATION
NIADDK, Bethesda, MD 20205

TOTAL MAN-YEARS: 0.0	PROFESSIONAL: 0.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Inactive.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM70507-02 CMOS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Alterations in Insulin's Action with Chronic Hyperinsulinemia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. B. Kahn	Medical Staff Fellow	CMOS, NIADDK
Others:	S. W. Cushman	Chief	CMOS, NIADDK
	D. R. Yver	Chemist	CMOS, NIADDK
	M. J. Zarnowski	Biologist	CMOS, NIADDK

COOPERATING UNITS *(if any)*

Metabolic Unit, Department of Medicine, University of Vermont College of Medicine, Burlington, VT (L. J. Wardzala, E. S. Horton).

LAB/BRANCH

SECTION

Cellular Metabolism and Obesity

INSTITUTE AND LOCATION

NIADDK, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The effects of chronic insulin administration on the metabolism of isolated rat adipose cells were studied. After 2 weeks of treatment, adipose cell size, and basal glucose transport and metabolism are unaltered, but insulin-stimulated glucose transport and metabolism are increased two- to three-fold. Insulin binding is increased 30-50%, but no shift in the insulin dose-response curve for glucose transport or metabolism occurs. Chronic hyperinsulinemia does not alter basal or epinephrine-stimulated lipolysis. These results suggest that chronic hyperinsulinemia increases insulin binding and the capacity of rat adipose cells to transport and metabolize glucose without changing the cells' sensitivity to insulin. The mechanism of increased insulin-stimulated glucose transport in adipocytes from chronically hyperinsulinemic rats was also examined. Adipocytes from insulin-treated rats show a 55% increase in insulin-stimulated glucose transport activity compared to control rats and a corresponding increase in glucose transporters in the plasma membranes. Basal glucose transport activity and glucose transporters in the plasma membranes were not significantly different. Basal and insulin-stimulated glucose transporters in the low-density microsomes were also unchanged. However, total intracellular glucose transporters in the basal state and the net translocation of intracellular glucose transporters to the plasma membranes in response to insulin were increased because of a 39% increase in low-density microsomal protein. Total protein and water space per cell were also increased in insulin-treated, compared to control, rats. These results suggest that chronic hyperinsulinemia in the rat enhances insulin's stimulatory action on glucose transport in adipocytes by increasing the intracellular pool of glucose transporters through a generalized effect on the net synthesis of intracellular protein.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM70508-02 CMOS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Insulin's Regulation of Hormone Binding

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	K. C. Appell	Staff Fellow	CMOS, NIADDK
Others:	I. A. Simpson	Visiting Scientist	CMOS, NIADDK
	S. W. Cushman	Chief	CMOS, NIADDK
	M. M. Rechler	Medical Officer	LBP, NIADDK
	M. J. Zarnowski	Biologist	CMOS, NIADDK

COOPERATING UNITS *(if any)*

Metabolic Unit, Department of Medicine, University of Vermont College Medicine, Burlington, VT (L. J. Wardzala).

LAB/BRANCH

SECTION

Cellular Metabolism and Obesity

INSTITUTE AND LOCATION

NIADDK, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previous studies have proposed that insulin increases the binding of insulin-like growth factor II (IGF-II) in isolated rat adipose cells by increasing receptor affinity (K_a). This study re-examines these observations under conditions in which receptor-ligand internalization is blocked by 1 mM KCN. In the absence of KCN, adipose cells bind 0.71 amol of IGF-II/cell with low apparent affinity, of which > 75% is not accessible to trypsin. In contrast, in the presence of KCN, IGF-II binding is decreased by 95% and its apparent affinity increased. Moreover, >60% of the bound IGF-II now is sensitive to trypsin. In either the absence or presence of KCN, ~ 20% of the cell's total IGF-II receptors are present in the plasma membranes and ~ 80% in the low-density microsomes. Insulin induces a 5-fold increase in cell surface IGF-II receptors without a change in affinity when IGF-II binding is measured in the presence of KCN. Similarly, insulin increases IGF-II receptor concentration in the plasma membranes and concomitantly decreases that in the low-density microsomes. Receptor affinity in these two subcellular membrane fractions is not affected by incubation of intact cells with either insulin or KCN and is similar to that observed in intact cells in the presence of KCN. Addition of KCN prior to insulin abolishes all of these effects of insulin. These data suggest that (a) the effects of KCN reflect a selective blockade of endocytosis; (b) in the absence of KCN, IGF-II binds to receptors of constant affinity that cycle between the plasma membrane and an intracellular pool resulting in an accumulation of intracellular IGF-II; (c) insulin induces an increase in IGF-II binding by causing a steady state redistribution of receptors from this intracellular pool to the plasma membrane; and (d) this redistribution in the intact cell can only be detected using Scatchard analysis when recycling of the receptors is prevented by KCN.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM70509-01 CMOS

PERIOD COVERED

October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Counterregulation of Insulin's Action by Catecholamines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Kuroda	Guest Worker	CMOS, NIADDK
Others:	I. A. Simpson	Visiting Scientist	CMOS, NIADDK
	S. W. Cushman	Chief	CMOS, NIADDK
	R. Honnor	Visiting Fellow	LCDB, NIADDK
	C. Londos	Research Chemist	LCDB, NIADDK
	M. J. Zarnowski	Biologist	CMOS, NIADDK

COOPERATING UNITS *(if any)*

Department of Medicine, University of Lund at Malmo, Malmo, Sweden (U. Smith)

LAB/BRANCH

SECTION

Cellular Metabolism and Obesity

INSTITUTE AND LOCATION

NIADDK, Bethesda, MD 20205

TOTAL MAN-YEARS: 1.6

PROFESSIONAL: 1.6

OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The interaction between catecholamines and insulin in regulating glucose transport in isolated rat adipose cells has been evaluated. In the presence of insulin, both isoproterenol and adenosine deaminase alone inhibit glucose transport activity ~ 25%. However, only the latter is accompanied by a corresponding decrease in the insulin-stimulated concentration of plasma membrane glucose transporters. Together, isoproterenol and adenosine deaminase inhibit insulin-stimulated glucose transport activity ~ 75%. However, these agents decrease the insulin-stimulated concentration of plasma membrane glucose transporters only ~ 45%. These results suggest that catecholamines counterregulate insulin-stimulated glucose transport in rat adipose cells through a cAMP-mediated mechanism, but only in part by modulating the translocation of glucose transporters. The mechanism of isoproterenol action has been further examined by measuring the protein kinase activity ratio, a marker of intracellular cAMP concentrations. 1) Adenosine deaminase alone decreases insulin-stimulated glucose transport activity by 30% without a change in the protein kinase activity ratio. Isoproterenol alone increases the protein kinase activity ratio without a change in insulin-stimulated glucose transport activity. Thus, under these conditions, changes in glucose transport activity do not correlate with the protein kinase activity ratio. However, 2) adenosine deaminase and isoproterenol in combination act synergistically to markedly increase the protein kinase activity ratio and markedly inhibit glucose transport activity. In conclusion, the counterregulation of insulin-stimulated glucose transport activity by isoproterenol appears to comprise both cAMP-independent and -dependent mechanisms.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AM70510-01 CMOS

PERIOD COVERED
October 1, 1983 to September 30, 1984

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Alterations in Insulin's Action with Fasting/Refeeding

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	B. B. Kahn	Medical Staff Fellow	CMOS, NIADDK
Others:	S. W. Cushman	Chief	CMOS, NIADDK
	D. R. Yver	Chemist	CMOS, NIADDK
	M. J. Zarnowski	Biologist	CMOS, NIADDK

COOPERATING UNITS *(if any)*

LAB/BRANCH

SECTION
Cellular Metabolism and Obesity

INSTITUTE AND LOCATION
NIADDK, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Rapid alterations in glucose transport and metabolism have been shown in rat adipocytes after fasting and refeeding. The mechanism for this was examined in rats fasted for 48 h and sacrificed ± 6d of refeeding. Adipocytes from fasted rats show a 67% decrease in insulin-stimulated glucose transport activity compared to nonfasted control rats and a corresponding decrease in glucose transporters in the plasma membranes. Fasting is associated with fewer glucose transporters in the basal low-density microsomes and a diminished translocation of glucose transporters from the low-density microsomes to the plasma membranes with insulin. Adipocytes from the refed rats show a 49% overshoot in insulin-stimulated glucose transport activity compared to the control rats with only a 13% increase in glucose transporters in the plasma membranes. The concentration of basal low-density microsomal glucose transporters appears lower in the refed rats compared to the control rats but total intracellular glucose transporters were increased due to a 53% increase in low-density microsomal protein. These results suggest that insulin resistance at the glucose transport level induced by fasting is due to a depletion of intracellular glucose transporters. In contrast, the hyperresponsive insulin-stimulated glucose transport activity associated with refeeding is not totally accounted for by a change in the number of glucose transporters and may also involve modulation of glucose transporter intrinsic activity.

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